Cucurbit Pollination: Mechanisms and Management to Optimise Field Quality and Quantity

Submitted by Jessica Louise Knapp, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences, May 2018.

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To my mum and dad

Frontispiece: Two *Bombus terrestris* L. sharing a staminate courgette (*Cucurbita Pepo* L.) flower. Photograph taken by Daphne Wong.

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Firstly, I would like to thank my supervisor, Juliet Osborne for being such a fantastic mentor over these last four years. Juliet's unwavering encouragement and kindness has been instrumental in helping me realise my potential and has made completing this PhD a real pleasure.

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Thesis abstract

Negative impacts from intensifying agriculture have generated concerns that pollinator-dependent crop species, such as courgette *Cucurbita pepo* L., may be experiencing a pollination deficit. This thesis explores the extent to which pollination influences fruit set; how pollination could be improved; and how in doing so growers' profits and agricultural resilience could increase, using UK field-grown courgettes as a model system.

Inspired by evidence of parthenocarpy (fruit set in the absence of fertilisation) in courgette, a systematic review showed extensive use of parthenocarpy to circumvent the need for pollination in other 'pollinator-dependent' crop species across the globe. Nonetheless, pollination significantly increased yield and pollinators were abundant enough to fulfil the pollination requirements of courgette, which if extrapolated to the rest of the UK, equates to pollinators contributing approximately £2.7 million to annual UK courgette production. Furthermore, wild flowers within fields were shown to be effective at increasing the abundance of bumblebees and solitary bees. Further exploration of the mutualistic relationship between courgettes and pollinators showed that courgette can improve populations of *Bombus terrestris* (using the *Bumble*-BEEHAVE model), an important pollinator of courgette.

This thesis concludes that pollination is a vital mechanism for ensuring optimal courgette yields and that whilst pollination levels were maximal at study sites, simple management, such as encouraging wild flowers within courgette fields could help to attract pollinators to courgette flowers and support bees' nutritional requirements beyond the extensive, yet transient, resource provided by courgette. Understanding a crop's requirement for pollinators can also aid

growers in their decision making about what varieties and sites should be used which could increase their agricultural resilience and further their economic advantage. Further work is needed to understand how other environmental factors interact with pollination to influence fruit set so that growers can prioritise key regulating services in their management for optimal crop yields.

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Publications

As a product of this thesis the following publications have arisen and are presented in Chapters 2, 3, and 4:

- Knapp, J.L., Bartlett, L.J. & Osborne, J.L. (2016) Re-evaluating strategies for pollinator-dependent crops: How useful is parthenocarpy? Journal of Applied Ecology, 1–9.
- Knapp, J.L. & Osborne, J.L. (2017) Courgette Production: Pollination Demand, Supply, and Value. Journal of Economic Entomology, 1–7.
- Knapp, J.L., Shaw, R. & Osborne, J.L. (2018) Pollinator visitation to mass-flowering courgette and co-flowering wild flowers: implications for pollination and pollinator conservation. Basic and Applied Ecology - Under Review.

Author's declaration

I am the main contributor of all the research presented in this thesis. Primary supervision came from Professor Juliet Osborne who contributed to the experimental design, analysis, and interpretation of results and provided feedback on all manuscripts. Further supervision came from Dr Rosalind Shaw in Chapter 4 and Dr Matthias Becher in Chapter 5, who both provided help with analysis, interpretation of results and feedback on the manuscripts. Lewis Bartlett contributed to the analysis and interpretation of results and provided feedback for Chapter 2. Under my supervision, Bethany Hasell and Jodie Henderson (Chapter 4), and Charlotte Rankin (Chapter 5) helped with data collection for their undergraduate theses. Additional help with data collection was provided by Ellie Brown for Chapters 4 and 5, and Louise Johns for Chapter 5.

Index of terms

Throughout this thesis I refer to crop species by their common name. This is because there are many different types of 'crops' within a single species, thus common names better reflect key differences (which are more obvious than variety differences) within species. Latin names are used for pollinator species throughout.

Term	Definition
Courgette	*Cucurbita pepo* Tosca is used as the main courgette variety throughout this thesis
Cucumber	*Cucumis sativus*
Farm-scale	Used to describe landscape-scale factors (outside of a study field) which may influence pollinators
Field-scale	Used to describe local-scale (within a study field) factors which may influence pollinators
Parthenocarpy	Fruit set in the absence of fertilisation and therefore pollination
Pumpkin	*Cucurbita moschata* Pumpkin is often used as a general term for summer and winter squashes. Where this is the case I have used the scientific name from the publication to determine the common name as defined by Kumar (2016).
Summer squash	*Cucurbita pepo* n.b. courgette is also *C. pepo*

Visitation to	The number of bees observed at courgette flowers, summed across transects (Chapters 4 and 5)
Visitation rate	Number of visits per minute per flower (Chapter 3)
Winter squash	*Cucurbita maxima*

1. Thesis introduction

Plate 1 *B. terrestris* visiting a pistillate courgette flower for nectar. Photograph taken by Daphne Wong.

Optimising ecosystem services

Globally, agricultural land is continuing to expand and intensify to meet rising food demands (Bommarco, Kleijn and Potts, 2013). Although successful for increasing yields, the loss and/or simplification of natural habitats combined with the replacement of many biological functions with artificial inputs has negatively affected the resilience and productivity of agricultural systems (Hooper, Chapin III and Ewel, 2005; Bommarco, Kleijn and Potts, 2013). Sustainable management of agricultural systems requires artificial inputs to be replaced or complemented with regulating and supporting ecosystem service management or 'environmentally-friendly' practices to enhance crop productivity (Tilman *et al.*, 2002; Bommarco, Kleijn and Potts, 2013). In doing so, producers can improve yields (Bommarco, Kleijn and Potts, 2013) and minimise negative impacts from intensive agriculture such as: habitat loss, nutrient runoff, and pesticide poisoning of non-target species (Tilman *et al.* 2002; Zhang *et al.* 2007; Power 2010; Pretty & Bharucha 2014).

Insect-mediated pollination (the transfer of pollen within or between flowers via an insect) is a key regulating service for many crops and wild plants; with 75% of global crop plants requiring insect pollination for fruit set, a figure which contains some of the most nutritional and culturally important components of human diet (Klein *et al.*, 2007). Indeed worldwide pollination services are estimated to be worth $153 billion, representing 9.5% of worldwide agricultural production used for human food (Gallai *et al.*, 2009). However, observed losses of pollinator populations combined with our dependence on their contribution to food security, has led to a widespread concern that we are facing a 'pollinator crisis' (Steffan-Dewenter *et al.* 2005; Potts *et al.* 2010; although see Ghazoul 2005). This has been exacerbated by a disproportionate increase in the area of

land producing pollinator-dependent crops (relative to nondependent crops); increasing the demand for pollination services (Aizen *et al.*, 2008).

To this end crop producers frequently rely on managed pollinator species to fulfil their pollination needs (Mader, Spivak and Evans, 2010). Increasing the abundance of species such as *Apis mellifera* L. (the Western honeybee) can interrupt the damaging cycle of lower yields resulting from a reduced abundance and species richness of wild pollinators, often caused by losses in (semi-) natural habitat (Garibaldi *et al.*, 2011). This is because wild and managed bee populations are limited by the abundance, diversity, and proximity to food and nesting sites which can be provided in (semi-) natural habitat (Roulston and Goodell, 2011). At a field scale, floral resources can be enhanced by planting wild flower strips, allowing areas to be naturally colonised by wild flowers, and maintaining floriferous hedgerows. At a farm scale, proximity to, or quantity of natural and semi-natural habitat can increase pollinator abundance as they spill into crop areas (Garibaldi *et al.*, 2011). Nonetheless, the effectiveness of pollinator-supporting practices are often variable and greatly depend on the complexity of the habitat surrounding a crop field, with more simplistic landscapes generally showing greater increases in pollinator abundance following management interventions than more complex landscapes (Batáry, Báldi, Kleijn, & Tscharntke, 2011; Scheper *et al.*, 2013; Herbertsson *et al.*, 2018).

Indeed, nectar and pollen from pollinator-dependent crop flowers can also provide pollinators with a substantial source of food (Westphal, Steffan-Dewenter and Tscharntke, 2003; Holzschuh *et al.*, 2013, 2016; Bailes *et al.*, 2015) (see Chapter 5). This mutualistic relationship means that crop flowers can

directly influence their own pollination success. In the short term, pollinators may be transiently attracted into the crop, enhancing their densities at crop flowers (Holzschuh *et al.*, 2016). Whilst in the longer term, phenological matching of crop flowering and pollinator activity could increase colony establishment and development (Bailes *et al.* 2015; although see Holzschuh *et al.* 2016). Interestingly, manipulating floral rewards to encourage pollinator visitation via selective breeding, has generally received little attention (Bailes *et al.*, 2015).

Measuring pollination success

In order to target pollinator management to species most likely to increase yield, much research has focused on quantifying pollinator performance in crops (Rader *et al.*, 2016). Broadly, there are two approaches for quantifying species-level pollinator performance: the first estimates pollinator behaviour and/ or pollen deposition on stigmas (Chapter 5), whilst the second estimates the pollinator's contribution to yield, usually measured as seed set or fruit weight (Chapter 3) (Ne'eman *et al.*, 2010). Arguably, when other environmental factors which influence fruit production e.g. soil type and cultivation practices cannot be standardised, single visit pollen deposition may be the most direct measure of pollination success (Kremen *et al.*, 2004). However, species-level effectiveness does not take into account the effectiveness of an entire pollinator community for a plant species in a given space or time (Willcox *et al.*, 2017). Thus, in Chapters 3 and 5 the contribution of all species to courgette fruit set was studied. For example, Willcox *et al.* (2017) identify that competitive or facilitative pollinator interactions and/or conspecific or heterospecific pollen transfer, observed by studying community-level effectiveness, may separately (or in combination) affect a plant's reproductive success.

Cucurbits

Cucurbitaceae (Cucurbits or gourds) are a large plant family which include major food plants such as *Cucurbita* (squash, pumpkin, courgette), *Cucumis* (cucumber, melon), and *Citrullus* (watermelon) (Kumar, 2016). Over centuries cucurbits have been domesticated for their fleshy fruits, roots, leaves, shoots, seeds and flowers for food and commodity goods and are therefore, economically important crops (Bates, Robinson and Jeffrey, 1990). Cultivated cucurbits can be grown in a variety of agricultural environments; from widespread monocultures to small-scale, traditional garden systems and many are able to persist in environmental conditions usually considered marginal for agriculture (Bates, Robinson and Jeffrey, 1990).

From a biological viewpoint, cucurbits' co-evolution with insects has provided much scientific intrigue. For example, their ability to produce bitter cucurbitacins has led to research into whether or not these compounds can be used for biological control, particularly against beetles (Metcalf *et al.*, 1982). Likewise, cucurbits' dependency on pollination (Free, 1993) means cucurbit flowers offer large quantities of nectar and pollen as floral rewards to visiting insects such as solitary bees, bumblebees and honeybees (Tepedino, 1981). In particular, the North American squash and gourd bees belonging to the genera *Peponapis* (Plate 2) and *Xenoglossa* are thought to rely exclusively on *Cucurbita* pollen to rear their offspring (Hurd, Linsley & Michelbacher 1974; Tepedino 1981).

Plate 2 *P. pruinosa* visiting a staminate courgette flower for pollen in California, USA.

From an agricultural viewpoint, various mechanisms have been explored to improve cucurbit yield such as eliminating dioecy (Boualem *et al.*, 2015), improving sex expression of flowers (Rodriguez-Granados *et al.*, 2017), and producing F_1 hybrid seed (Robinson, 2000). Indeed, the yield (per hectare) of cucurbit crops has steadily increased over the last 50 years particularly in Asia where pioneering technological advancements and genetic improvements, especially with seedless varieties, have advanced cucurbit production worldwide (Figure 1.1~~Figure 1.1~~) (McCreight *et al.*, 2013). Likewise, and most relevant to this thesis, cucurbit yield can also be increased by improving the level of pollination (Hoehn *et al.*, 2008; Kouonon *et al.*, 2009).

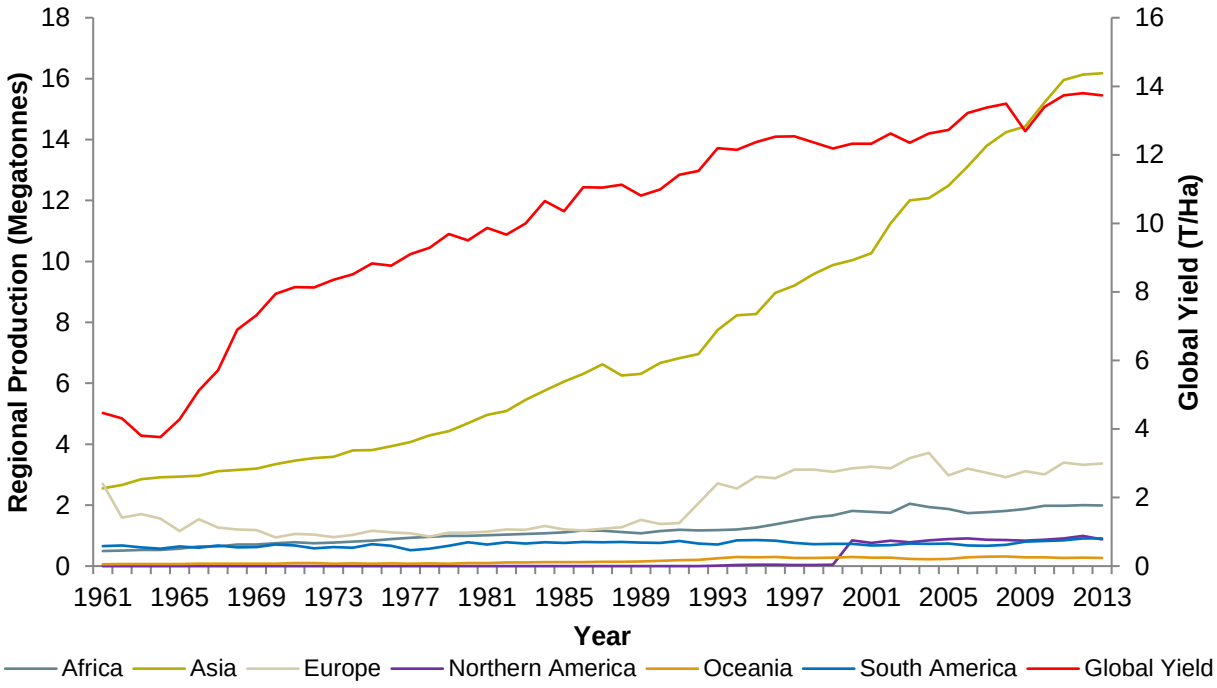


Figure 1.1 Regional production (primary y axis) and average global yield (secondary y axis) of *Cucurbita* species from 1961 to 2013. Data source: FAOSTAT (Aggregate, may include official, semi-official, estimated or calculated data).

Mechanism of cucurbit pollination

Pollinator dependency

Cucurbits are described as having an 'essential need' for insect-mediated pollination (Free, 1993; Klein *et al.*, 2007). This assertion is based on previous research which has shown that seed number (Roldán-Serrano and Guerra-Sanz, 2005) and fruit set of courgette (Roldán-Serrano and Guerra-Sanz, 2005) and summer squash (*Cucurbita pepo* L., Vidal *et al.* 2010) and fruit set of cucumber (*Cucumis sativus* L., Gingras, Gingras & DeOliveira 1999; Walters 2005) are positively correlated to the number of pollinator visits. Similarly, fruit has been shown to abort in the absence of pollination in cucumber (Motzke *et al.*, 2015), melon (*Cucumis melo* L., Kouonon *et al.* 2009), pumpkin (*Cucurbita moschata* Duch. ex Poir., Hoehn *et al.* 2008), and courgette (*Cucurbita pepo,*

Martínez *et al.* 2014). Interestingly, fruit development of small-sized summer squash varieties was not as influenced as larger ones by the addition of honey bee colonies; suggesting that some smaller varieties may be able to set fruit with a smaller pollen load (Walters and Taylor, 2006).

However, under certain conditions some cucurbit varieties may be able to set without fertilisation, and therefore without pollination, via a process called parthenocarpy (Figure 1.2~~Figure 1.2~~). The ability of a plant to set fruit without pollination has long been recognised as a desirable characteristic for greenhouse-grown cucumber which has led to an extensive selective breeding program for this genetic trait (Robinson and Reiners, 1999). Whilst other cucurbit species have generally received less attention, several varieties of summer squash have been observed to set fruit without any pollination (Robinson and Reiners, 1999; Kurtar, 2003; Martínez *et al.*, 2013). However, evidence suggests that parthenocarpic varieties may still produce a greater quantity and quality, including a higher sugar content (Shin, Park and Kim, 2007) of fruits when pollinated by insects (Martínez *et al.* 2013; Robinson & Reiners 1999; Nicodemo *et al.* 2013). A meta-analysis of parthenocarpy in crop species is presented in Chapter 2.

Pollinator efficiency

In North America research has shown *Peponapis* spp. to be highly abundant (Tepedino, 1981), effective pollinators of *Cucurbita* crops, depositing more pollen grains per stigma and visiting crop flowers more frequently than *A. mellifera* (Canto-aguilar and Veterinaria, 2000). Likewise, Artz and Nault (2011) observed *Bombus impatiens* C. (another North American species) to be a highly effective pollinator in summer squash, depositing more than three times the

number of pollen grains per stigma and nearly always contacting the stigma compared to *A. mellifera* and *Peponapis pruinosa* S,. Consequently, in small fields (0.5 ha) with managed *B. impatiens* colonies present, fruit yield was nearly twice that of non-supplemented fields (Table 1.1~~Table 1.1~~). *B. impatiens* have also been observed to set more seeds per fruit than *A. mellifera* in watermelon (Stanghellini, Ambrose and Schultheis, 1998). Nonetheless, *A. mellifera* has been observed to spend two to three times' longer handling summer squash flowers than *B. impatiens* and *P. pruinosa* respectively (Artz and Nault, 2011). However, *A. mellifera* appears to significantly favour pistillate cucurbit flowers (Tepedino, 1981; Artz, Hsu and Nault, 2011; Phillips and Gardiner, 2015), unlike species such as *P. pruinosa* whose proportion of visits more closely resemble the natural sex ratio of cucurbit flowers (Artz, Hsu and Nault, 2011; Phillips and Gardiner, 2015).

Although these studies demonstrate that several species can be highly effective pollinators (See Chapters 3, 4 and 5), some evidence suggests that a diverse assemblage of species is required for optimum fruit set. For example, a diverse assemblage of pollinators have been observed to visit melon, watermelon (Ali *et al.*, 2015), and summer squash (Ali *et al.*, 2014) and species diversity has been shown to increase seed set in pumpkin (Hoehn *et al.*, 2008). Likewise, Pisanty *et al.* (2015) observed spatial and temporal variation in pollinator visitation to watermelon, suggesting niche complementarity.

Management for pollination in cucurbit crops

Pollination deficit

Although some researchers have addressed the extent of cucurbits' dependency on pollinators and pollinator efficiency in cucurbits, relatively few,

and none outside of the United States, have looked at if cucurbit crops are experiencing a pollination deficit (Chapter 3), i.e. if crop quality and quantity could be improved with more pollination (Garratt *et al.*, 2013).

Walters and Taylor (2006) showed that adding colonies of *A. mellifera* to fields of winter squash increased fruit weight (per hectare) by 100%. However, similar studies have shown that supplementing fields with *B. impatiens* and *A. mellifera* did not increase pollinator visitation to, or yield of summer squash (Petersen, Reiners and Nault, 2013). Likewise, introduced *B. impatiens* did not influence fruit weight, seed set and visitation in summer squash and there was no difference in yield between open- and hand-pollinated flowers (Petersen, Huseth and Nault, 2014). In addition, several studies have found no evidence of increased *A. mellifera* visitation to *Cucurbita* species (Shuler, Roulston and Farris, 2005), despite fields having *A. mellifera* colonies added.

These results (summarised in Table 1.1~~Table 1.1~~) are most likely due to already high levels of open pollination at study sites, evidenced by high yields in control plots (Artz, Hsu and Nault, 2011; Petersen, Reiners and Nault, 2013; Petersen, Huseth and Nault, 2014). Since pollinator visitation positively influences yield, fruit set is directly dependent on pollinators and the ecosystems which support their populations. Therefore, these types of results are highly dependent on the spatial and temporal context of the landscape surrounding each crop field. Nonetheless, determining if a study site is experiencing a pollination deficit should be a vital step before implementing any management interventions to promote pollinator populations. This was nicely demonstrated by Julier & Roulston (2009) who combined pollinator visitation data at their study sites with published data on pollination requirements of summer squash to determine that

wild bee densities at their study sites were sufficient to fulfil their pollination needs. Pollination deficit in UK courgette crops is examined in Chapter 3.

Table 1.1 Effect of supplemented commercial pollinators on cucurbit yield and pollinator visits to cucurbit flowers.

Citation	Species	Commercial Pollinator	Effect	Dependent variable
Artz and Nault. 2011	*Cucurbita pepo*	*A. mellifera*	Positive effect	*A. mellifera* visits per flower
Artz and Nault. 2011	*Cucurbita pepo*	*A. mellifera*	No effect	*B. impatiens* visits per flower
Artz and Nault. 2011	*Cucurbita pepo*	*A. mellifera*	Negative effect	*P. pruinosa* visits per flower
Peterson *et al.* 2013	*Cucurbita pepo*	*A. mellifera*	No effect	Yield (fruit weight)
Walters *et al.* 2006	*Cucurbita pepo*	*A. mellifera*	No effect	Yield (fruit weight)
Walters *et al.* 2006	*Cucurbita moschata*	*A. mellifera*	Positive effect	Yield (fruit weight)
Walters *et al.* 2006	*Cucurbita maxima*	*A. mellifera*	Positive effect	Yield (fruit weight)
Peterson *et al.* 2014	*Cucurbita pepo*	*B. impatiens*	No effect	*B. impatiens* visits per flower
Peterson *et al.* 2013	*Cucurbita pepo*	*B. impatiens*	No effect	Yield (fruit weight)
Peterson *et al.* 2014	*Cucurbita pepo*	*B. impatiens*	No effect	Yield (fruit weight)
Artz and Nault. 2011	*Cucurbita pepo*	*B. impatiens*	Positive effect	Yield (number of fruits)
Peterson *et al.* 2014	*Cucurbita pepo*	*B. impatiens*	No effect	Yield (number of seeds)

Spatial scales of pollination management

Whilst managed species such as *A. mellifera* and *B. impatiens* can greatly enhance cucurbit yield, cucurbits could also experience greater yields in more diverse habitats (Chapter 4); where increased species richness and abundance of wild pollinators can improve pollination services (Hoehn *et al.*, 2008; Garibaldi *et al.*, 2011) and provide insurance against any pollinator loss (Shuler, Roulston and Farris, 2005). Improving the quantity and quality of pollen and nectar resources available for pollinators, and allowing areas to remain undisturbed for nesting, mating, and hibernation could benefit pollinator populations and therefore reduce pollination deficits (Bommarco, Kleijn and Potts, 2013). At a field scale (Table 1.2~~Table 1.2~~), wild flowers co-flowering with crops have been shown to increase solitary bee abundance in muskmelon and watermelon (Winfree *et al.* 2008). Since *P. pruinosa* preferentially lay their eggs in crop areas at depths around 12 to 30 cm (Julier & Roulston 2009; Hurd *et al.* 1974), no-tillage farms have been shown to have an almost three-fold increase in *P. pruinosa* density (Shuler, Roulston and Farris, 2005). However this evidence is conflicted by other studies which have observed no effect of tillage on *P. pruinosa* abundance (Julier & Roulston 2009), most likely due to different phenology of study crops, which were surveyed mid-August in Julier & Roulston (2009) compared to July (Shuler, Roulston and Farris, 2005). Further, *P. pruinosa* have been observed to emerge from heavily tilled and disturbed areas (Minter & Bessin 2014).

At a farm scale smaller crop fields and increased proximity to natural habitats can increase forage and nesting opportunities nearer to crops and is likely to be why species known for their longer flight distances such *A. mellifera* are found in high abundance in large, intensively farmed fields (Osborne, Martin, Carreck, *et al.* 2008).

Consequently, Kremen *et al.* (2004) found that pollination by native bees in watermelon was strongly associated with the proportion of natural habitat within a 1 to 2.5 km radius of a farm sites. This relationship was strong enough for the authors to suggest that based on the area of natural habitat, pollination services to a given area could be estimated (Kremen *et al.*, 2004). Although organic farming was shown to increase pollinator abundance, and therefore, reduce pollination deficit in oilseed rape (Morandin and Winston, 2005), similar findings have not been observed in cucurbits (Kremen *et al.* 2004; Winfree *et al.* 2008, Table 1.3). For example, organic (versus conventional) farming has been shown to be less important than the amount of natural habitat surrounding a study site for predicting pollen deposition (Kremen *et al.*, 2004), pollinator abundance (Kremen *et al.*, 2004; Winfree *et al.*, 2008), and species richness (Winfree *et al.*, 2008) in watermelon.

As many agricultural systems are isolated from natural habitats, crop producers may need to provide floral resources and nesting sites suitable for pollinators. In the UK, farm stewardship schemes provide guidance on hedgerow and field margin management, particularly favoured by bumblebee species (Osborne, Martin, Shortall, *et al.*, 2008; Carvell *et al.*, 2015; Dicks *et al.*, 2015; Wood, Holland and Goulson, 2015). Alternatively, costs can be directly offset by increased profit from improved quality and quantity of yields. For example, the economic benefit of improved blueberry yields following wild flower establishment has been shown to exceed the original cost of wild flower establishment (Blaauw and Isaacs, 2014). The effect of forage availability on pollinator abundance is explored in Chapter 4 and Chapter 5.

Table 1.2 Effect of field-scale pollinator management practices on pollinator visits to, and pollen deposition on cucurbit flowers.

Citation	Species	Agricultural Practice	Effect	Dependent variable
Phillips and Gardiner 2015	*Cucurbita pepo*	Floral strips	No effect	“Bee visitation frequency” per flower
Phillips and Gardiner 2015	*Cucurbita pepo*	Floral strips	No effect	Pollen deposition per flower
Shuler *et al.* 2005	*Cucurbita pepo*	Pesticide use	No effect	*Bombus* spp. visits per flower
Shuler *et al.* 2005	*Cucurbita pepo*	Pesticide use	No effect	*P. pruinosa* visits per flower
Julier and Roulston. 2009	*Cucurbita pepo*	Soil clay content	Negative effect	*P. pruinosa* abundance
Shuler *et al.* 2005	*Cucurbita pepo*	Tillage	No effect	*B. impatiens* visits per flower
Julier and Roulston. 2009	*Cucurbita pepo*	Tillage	No effect	*P. pruinosa* abundance
Shuler *et al.* 2005	*Cucurbita pepo*	Tillage	Positive effect	*P. pruinosa* visits per flower

Table 1.3 Effect of farm-scale pollinator management practices on cucurbit yield, pollinator visits to, and pollen deposition on cucurbit flowers.

Citation	Species	Landscape complexity	Effect	Dependent variable
Kremen *et al.* 2004	*Citrullus lanatus*	Organic farming	No effect	Pollen deposition per flower
Winfree *et al.* 2008	*Citrullus lanatus*	Organic farming	No effect	Bee abundance/ flower/ time
Winfree *et al.* 2008	*Cucumis melo*	Organic farming	No effect	Bee abundance/ flower/ time
Winfree *et al.* 2008	*Citrullus lanatus*	Woodland	Positive effect	Bee abundance/ flower/ time
Winfree *et al.* 2008	*Cucumis melo*	Woodland	No effect	Bee abundance/ flower/ time
Peterson and Nault. 2014	*Cucurbita pepo*	Landscape diversity	Positive effect	*A. mellifera* visitation frequency
Peterson and Nault. 2014	*Cucurbita pepo*	Landscape diversity	Positive effect	*B. impatiens* visitation frequency
Peterson and Nault. 2014	*Cucurbita pepo*	Landscape diversity	Positive effect	Yield (fruit weight)
Phillips and Gardiner 2015	*Cucurbita pepo*	Landscape diversity	Positive effect	“Bee visitation frequency”
Phillips and Gardiner 2015	*Cucurbita pepo*	Landscape diversity	Positive effect	Pollen deposition per flower
Julier and Roulston. 2009	*Cucurbita pepo*	Natural habitat	No effect	*P. pruinosa* abundance
Kremen *et al.* 2004	*Citrullus lanatus*	Natural habitat	Positive effect	Pollen deposition per flower
Peterson and Nault. 2014	*Cucurbita pepo*	Grassland	No effect	*A. mellifera* abundance per flower
Peterson and Nault. 2014	*Cucurbita pepo*	Grassland	Positive effect	*B. impatiens* abundance per flower
Peterson and Nault. 2014	*Cucurbita pepo*	Grassland	Positive effect	Yield (fruit weight)
Artz and Nault. 2011	*Cucurbita pepo*	Field size	No effect (but positively interacted with *A. mellifera* supplementation)	*B. impatiens, A. mellifera,* and *P. pruinosa* abundance
Kremen *et al.* 2004	*Citrullus lanatus*	Field size	No effect	Pollen deposition per flower

Pollination in context

Whilst pollination clearly affects cucurbit yield, there are many other environmental factors which contribute and interact with each other to influence fruit set (Figure 1.2~~Figure 1.2~~) such as soil quality, water availability and weather conditions (Boreux *et al.*, 2013; Klein *et al.*, 2014; Motzke *et al.*, 2015). For example, soil nitrogen has been shown to increase the number, weight, and viability of pollen grains, with pollen from plants grown in higher nitrogen environments observed to produce courgettes with more seeds (Lau Tak-Cheung and Stephenson, 1993). Likewise, Motzke *et al.* (2015) showed that weed control and fertilisation were able to reduce the yield gap of cucumbers by 45% and 18% respectively, however, these factors, even in combination, were unable to account for a total absence of pollination (increased yield gap of 75%).

Therefore, the productivity of pollinator-dependent crops relies on the presence of high functioning ecosystems to support pollinator populations, regulate disease, purify and cycle water and nutrients. Any impact (particularly anthropogenic (Winfree *et al.*, 2009)) on the wider ecosystem will have a detrimental impact on crop yields and farmers profits (Steffan-Dewenter *et al.*, 2005; Potts *et al.*, 2010; Goulson *et al.*, 2015). Thus whilst this thesis is principally concerned with the mechanism of cucurbit pollination and management for this service, other factors which may affect cucurbit yield (Figure 1.2~~Figure 1.2~~), although not directly studied, were still taken into account.

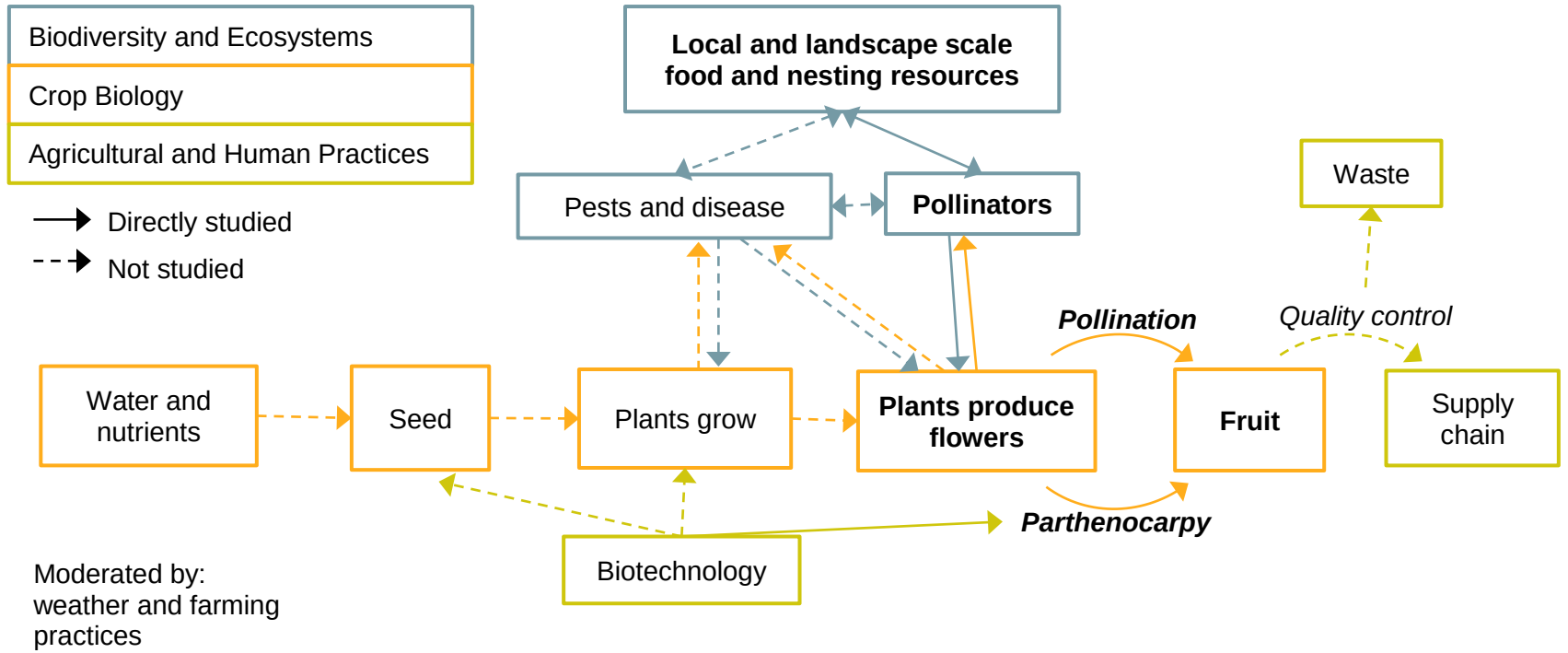


Figure 1.2 Conceptual framework of the different factors (broadly categorised into crop biology, agricultural and human practices, and biodiversity and ecosystems) which effect fruit set in pollinator-dependent crops. Solid lines and bold text show factors which were directly studied whilst dashed lines and plain text show factors which were considered but not directly studied in this thesis.

Project rationale

Funded by the Agriculture and Horticulture Development Board and in collaboration with courgette growers in the UK (Plate 3), this study is the first to explore cucurbit pollination in the United Kingdom. Consequently, no information was available prior to this study about how effective UK species were at pollinating cucurbits. Likewise, no work had been done on the pollinator dependence of "Tosca", a popular variety of courgette in the UK and the principle variety used in this study; whether these plants were experiencing a pollination deficit, and indeed, the economic value of pollination to UK courgette production. Based on this information floral resources influencing the most effective pollinators were explored, and the effect of cucurbit nectar and pollen on bumblebee population dynamics, using computer simulations was explored for the first time.

Accordingly, this research directly addresses priorities on the Outdoor Cucurbit Research and Development Priority List: "Pollination for fruit quality: supporting pollinating insects", and the Agriculture and Horticulture Development Board's Field Vegetable Sector Priority List: "To supply consistent quality product and continuity and to achieve customer satisfaction", as well as: "making efficient use of resources to improve returns".

Therefore, by understanding courgette pollination dynamics within the context of UK growing conditions, this PhD aims to give commercial growers management options that would allow them to improve the quantity and quality of their yield, supporting sustainability and profitability. These finding are also relevant to hobbyist growers as well as the wider public by promoting the value of pollination to horticultural crop production.

Plate 3 Courgette field being picked by Riveria Produce, a key collaborator of this PhD project, in Cornwall, UK. Photograph taken by Russell Barnett and Muddy Duck Productions.

Plate 4 Courgette field and surrounding landscape in Cornwall, UK. Photograph taken by Russell Barnett and Muddy Duck Productions.

Thesis overview

In this thesis I develop on these themes by addressing three key areas of courgette pollination, highlighted with solid arrows in Figure 1.2~~Figure 1.2~~. The first explores the mechanism, variability, and economic value of courgette pollination (Chapter 2 and Chapter 3). The second looks at ways of improving pollination within courgette fields (Chapter 4). Finally, the third explores the mutualism between courgette and its key pollinator species (Chapter 5). I have addressed these themes through a series of four complementary data chapters, a simple description of each, and the knowledge gaps addressed follows:

Chapter 2: Re-evaluating strategies for pollinator-dependent crops: how useful is parthenocarpy?

Whilst most studies reviewing the reliance of global agriculture on insect pollination advocate increasing the 'supply' of pollinators (wild or managed) to improve crop yields, there has been little focus on altering a crop's 'demand' for pollinators. Parthenocarpy (fruit set in the absence of fertilisation) is a trait which can increase fruit quantity and quality from pollinator-dependent crops by removing the need for pollination. Therefore, this meta-analysis asks how **extensive and effective are parthenocarpy-promoting techniques** (genetic modification, hormone application and selective breeding) for conferring fruit set in usually pollinator-dependent crops? This study highlights the potential that parthenocarpy could have to lower a crop's demand for pollinators, allowing growers to extend their current geographic and climatic ranges of production, ultimately improving food security and the economic prospects of growers. Published: Knapp, J.L., Bartlett, L.J. & Osborne, J.L. (2016) Re-evaluating

strategies for pollinator-dependent crops: How useful is parthenocarpy? Journal of Applied Ecology, 1–9.

Chapter 3: Courgette production: pollination demand, supply, and value

Courgette production in the UK is estimated to be worth £6.7 million. However, little is known about this crop's requirement for insect-mediated pollination (pollinator dependence) and if pollinator populations in the landscape are able to fulfil its pollination needs (pollination deficit). Consequently, pollination experiments were conducted to ask to what extent **does pollination influence fruit set and are field grown courgettes experiencing a pollination deficit?** Results are discussed in the context of the economic value of pollination to courgette production in the UK. Published: Knapp, J.L. & Osborne, J.L. (2017) Courgette Production: Pollination Demand, Supply, and Value. Journal of Economic Entomology, 1–7.

Chapter 4: Pollinator visitation to mass-flowering courgette and co-flowering wild flowers: implications for pollination and pollinator conservation

Allocation of floral resources to increase pollinator abundance is the primary basis for pollinator-supportive land management; however, relatively little is known about how the scale of floral resources (field or farm) may affect different pollinator groups which may or may not pollinate the focal crop. Therefore, this study asks **how do floral resources influence pollinator abundance in courgette fields?** By understanding this more fully, pollinator-supportive land management can be targeted either to pollinator species which are known to visit the focal crop, with the aim of improving crop pollination, or to other pollinator species more generally, with the aim of improving species conservation; or both. Under Review: Knapp, J.L., Shaw, R. & Osborne, J.L.

(2018) Pollinator visitation to mass-flowering courgette and co-flowering wild flowers: implications for pollination and pollinator conservation. Basic and Applied Ecology.

Chapter 5: *Bombus terrestris* in a mass-flowering pollinator-dependent crop: A mutualistic relationship?

Mass-flowering crops are frequently overlooked as a nutritional resource for bumblebees despite being the intended forage resource for growers wishing to obtain pollination services. This study explores the mutualistic relationship between *Bombus terrestris* and courgette by combining empirical data on pollination efficiency and nectar and pollen availability with model simulations using the novel bumblebee model *Bumble*-BEEHAVE, a new bee systems model (Becher, Matthias A. Twiston-Davies *et al.*, 2018). Therefore, this study asks **how effective is *B. terrestris* at pollinating courgette and in return, how does courgette affect *B. terrestris* colony dynamics?** Understanding how bumblebees utilise crop flowers i.e. only for nectar, only for pollen, or nectar and pollen will provide an insight into how likely colonies are to develop and how much pollination is likely to occur.

2. Re-evaluating strategies for pollinator-dependent crops: how useful is parthenocarpy?

Preliminary pollination experiments unexpectedly showed that courgette was able to set fruit without any pollination (Chapter 3). Therefore, to improve estimates of pollinator dependence in crops and to improve guidance to farmers for improving fruit production, I reviewed the literature for further evidence of this kind of fruit set in crop species with are usually considered to be dependent on pollinators for fruit set. Food security, particularly of pollinator-dependent crops is a current global concern, this work shows how environmental and technological solutions could and *should* be used in tandem to ensure the best possible crop yields where they are needed most.

Plate 5 Courgette fruit set following a 'no pollination treatment', initiated the day before expected anthesis by securing PVC mesh bags with wire ties to pistillate flowers. Results of this pollination experiment are further explored in Chapter 3.

Abstract

Whilst most studies reviewing the reliance of global agriculture on insect pollination advocate increasing the 'supply' of pollinators (wild or managed) to improve crop yields, there has been little focus on altering a crop's 'demand' for pollinators. Parthenocarpy (fruit set in the absence of fertilisation) is a trait which can increase fruit quantity and quality from pollinator-dependent crops by removing the need for pollination. Here we present a meta-analysis of studies examining the extent and effectiveness of parthenocarpy-promoting techniques (genetic modification, hormone application and selective breeding) currently being used commercially, or experimentally, on pollinator-dependent crops in different test environments (no pollination, hand pollination, open pollination). All techniques significantly increased fruit quantity and quality in 18 pollinator-dependent crop species (not including seed and nut crops as parthenocarpy causes seedlessness). The degree to which plants experienced pollen limitation in the different test environments could not be ascertained, so the absolute effect of parthenocarpy relative to optimal pollination could not be determined. Parthenocarpy has the potential to lower a crop's demand for pollinators, whilst extending current geographic and climatic ranges of production. Thus, growers may wish to use parthenocarpic crop plants, in combination with other environmentally considerate practices, to improve food security and their economic prospects.

Introduction

Globally, agricultural land is continuing to expand and agricultural practices continue to intensify to meet rising food demands (Bommarco, Kleijn and Potts, 2013). It is argued that sustainably maximising agricultural yield requires ecosystem services to be optimised through improved soil quality, water efficiency and management of beneficial insects for pest control and pollination (Tilman *et al.*, 2002; Bommarco, Kleijn and Potts, 2013). Insect-mediated pollination (the transfer of pollen within or between flowers via an insect) is a key regulating service for many crops and wild plants (Wilcock and Neiland, 2002; Klein *et al.*, 2007). Thus any detrimental impact on pollination services, for example from habitat loss, introduced pests and diseases, and practices associated with intensive agriculture, could have a negative effect on crop yields and farmers' profits (Steffan-Dewenter *et al.*, 2005; Potts *et al.*, 2010; Goulson *et al.*, 2015). Observed losses of pollinator species combined with our dependence on their contribution to food security, has led to a widespread concern that we are facing a 'pollinator crisis' (Steffan-Dewenter *et al.* 2002; Potts *et al.* 2010; although see Ghazoul 2005). However, whilst the plethora of recent reviews and studies on this subject come to similar conclusions that improving habitat and environmental conditions for pollinators will have a positive impact on crop production by increasing the 'supply' of pollinators (wild or managed); none of these studies consider the alternative option of reducing 'demand' for crop pollinators via technological innovation or management of crops. This can lead to a narrow (and potentially out-dated) perspective given that, in the meantime, plant breeders and farmers are finding ways of short-circuiting the need for pollination by developing and using new varieties which can set fruit without pollen vectors (Pandolfini, Molesini and Spena, 2009).

The need for insect pollination in crops is usually measured in two ways: 1) **Pollinator dependence** is quantified by comparing the yield of open-or hand-pollinated crops with the yield of crops from which pollinators have been excluded. However, this is often only done for single cultivars in particular environmental conditions (Chapter 3); 2) **Pollination deficit** estimates the additional pollination needed to achieve maximum yields in a particular context by comparing open-pollinated with hand-pollinated crops (Vaissière, 2010). This technique has identified pollination deficits in a range of pollinator-dependent crop species and is a vital step to evidence the need to implement management interventions to promote pollinator populations. Realistic estimates of the 'value' of insect pollination to global agriculture need to account for not only the variability in pollination deficit that might result from variable pollinator densities and environmental conditions, but also the variability in pollinator dependence between varieties of single crop species, for which there is currently little good evidence (Melathopoulos, Cutler and Tyedmers, 2015). In the wider context, discussion and strategies for improving horticultural crop production (in particular) need to incorporate evidence on the variety of options available for increasing fruit and seed set by manipulating pollination systems, and not just assume that the only way to do this is by maximising pollination. To improve estimates of pollinator dependence in crops, and to widen the debate about how to guide farmers in improving seed and fruit production, we present a meta-analysis of studies inducing **parthenocarpy** in horticultural crops.

Parthenocarpy (fruit set in the absence of fertilisation) is a trait which has the potential to make many 'pollinator-dependent' species produce fruit without pollination (Vardi, Levin and Carmi, 2008). Parthenocarpy is thought to increase fruit quantity as plants are able to set fruit in conditions adverse for fertilisation,

for example due to poor pollen maturation or few pollinating species, typically seen in greenhouses or during periods of poor light and cold temperatures (Pandolfini, 2009). Without parthenocarpy, and under these conditions, growers would ordinarily experience high rates of fruit abortion due to an insufficient number of pollen grains delivered to stigmas (Pandolfini, 2009).

Parthenocarpy also has the potential to improve fruit quality as seedlessness (caused by no pollination and therefore fertilisation) can be a desirable trait. This is different to stenospermocarpy, where seedlessness is achieved by seeds being aborted after fertilisation (and therefore pollination) such as with triploid watermelons (Varoquaux *et al.*, 2000). For example, it is thought to extend shelf-life in some species, such as reduced browning in aubergine (Acciarri *et al.*, 2002), is advantageous in fruit processing, such as tinned tomatoes (Pandolfini *et al.*, 2002), and is generally favoured by consumers for convenience in preparation and consumption (Vardi, Levin and Carmi, 2008). However, evidence suggests that some parthenocarpic plants may still produce a greater quantity and quality (including higher sugar content (Hayata *et al.*, 2000; Shin, Park and Kim, 2007)) of fruits when pollinated by insects (Robinson and Reiners, 1999; Martínez *et al.*, 2013; Nicodemo *et al.*, 2013).

Fertilisation of the ovules and seed/ fruit development is co-ordinated by various phytohormones, including auxins, gibberellins, and cytokinins which originate from the developing embryos (Gillaspy, Ben-David and Gruissem, 1993). Phytohormones, present in developing seeds are vital for regulating fruit growth and development (Gillaspy, Ben-David and Gruissem, 1993). However, in parthenocarpic (and therefore seedless) fruit set, endogenous phytohormones are elevated, suggesting that phytohormones from sources other than

developing seeds can regulate fruit growth (Gustafson, 1936). Consequently, parthenocarpy may be initiated through exogenous application of phytohormones. Auxins, gibberellins, and cytokinins or mixtures of these have all been proven to be effective in inducing fruit development in the absence of fertilization and have been shown to increase productivity in various horticultural crops (Reviewed in Pandolfini 2009). However, little is known about the effect of these hormones on the environment and implementation is expensive and labour-intensive (Saito *et al.*, 2009). Consequently, scientists are increasingly finding ways to exploit genetic parthenocarpy.

Traditionally, approaches to genetic parthenocarpy have largely focused on selective breeding programs for seedlessness (reviewed in Vardi *et al.* 2008 and Varoquaux *et al.* 2000). For example, selective breeding of parthenocarpic sweet pepper (Tiwari, Dassen and Heuvelink, 2007; Honda *et al.*, 2012), papaya (Rimberia *et al.*, 2007), and summer squash (Robinson and Reiners, 1999; Kurtar, 2003) varieties have all been shown to increase productivity. More recently, scientists have focused on genetic engineering approaches for parthenocarpic fruit set, through modification of auxin synthesis (iaaM), auxin sensitivity (rolB), auxin content (Aucsia), auxin signal transduction (iAA9 or ARF8), and gibberellin signal transduction (DELLA) (reviewed in Pandolfini 2009). For example, the chimeric auxin synthesising DefH9-iaaM gene has been shown to increase productivity in aubergine (Rotino *et al.*, 1997; Donzella, Spena and Rotino, 2000; Acciarri *et al.*, 2002), tomato (Pandolfini *et al.*, 2002; Molesini *et al.*, 2009), cucumber (Yin *et al.*, 2006), strawberry (Mezzetti *et al.*, 2004), and raspberry (Mezzetti *et al.*, 2004). Auxin-synthesis parthenocarpy is facultative, meaning that it is seedless in conditions adverse for pollination/ fertilisation and seeded (although much reduced in number (Rotino *et al.*,

2005)) in conditions where pollination occurs (Pandolfini, Molesini and Spena, 2009). Breeding for genetic parthenocarpy also has the potential to combine multiple desirable traits. For example, parthenocarpy, pistillate-flowering time, improved fruit quality and disease resistance have been combined in cucumbers (Kushnereva, 2008).

Using parthenocarpy to promote fruit set under unfavourable environmental conditions could improve the quality and quantity of pollinator-dependent crops by reducing the number of poorly formed fruits caused by insufficient pollination (Pandolfini, 2009). This could extend current geographic and climatic agricultural ranges of production, simultaneously improving food security and the economic prospects of commercial growers. Methods to induce parthenocarpy should therefore be considered when calculating the contribution of pollinators to fruit set, to avoid over-estimating our dependence on them. Klein *et al.* (2007) provide the most comprehensive review of global crop pollinator dependence, and they acknowledge that their results are often based on studies from single cultivars and/or single regions because of the difficulty of finding comprehensive evidence. However, their data have been used to subsequently estimate the global value of pollination (Gallai *et al.*, 2009; Breeze *et al.*, 2011) and consequently justify the prediction of a 'pollination crisis'(Steffan-Dewenter *et al.*, 2005; Potts *et al.*, 2010) without substantiated information at the individual crop level, as highlighted by Melathopolous *et al.* (2015).

In this paper we aim to go beyond previous reviews of parthenocarpy (Varoquaux *et al.*, 2000; Gorguet, Van Heusden and Lindhout, 2005; Vardi, Levin and Carmi, 2008; Pandolfini, 2009; Pandolfini, Molesini and Spena, 2009)

by using meta-analysis techniques to review and synthesise the literature on the extent of parthenocarpy promoting techniques currently being used commercially or experimentally on pollinator-dependent crops across the world. Systematically reviewing plant science literature and applying it to pollination biology provides a broader perspective on the pollinator debate. We specifically investigate the following questions: (1) Does artificial or genetic parthenocarpy increase the quantity and quality of fruits in (normally) pollinator-dependent crop species? (2) Which method for conferring parthenocarpy: selective breeding, genetic modification or growth hormones, is most effective for parthenocarpic fruit set?

Methods

Data Collection

We searched the ISI Web of Science, SCOPUS, Science Direct, Directory of Open Access Journals, AGRICOLA databases, and, Google Scholar, for studies that investigated the effect of genetic and artificial parthenocarpy on the quantity or quality of yield in pollinator-dependent crops as defined by Klein *et al.* (2007), where pollinator dependence is classified as 'essential', 'great', 'modest' or 'little' (Table A 2.1~~Table A 2.1~~). Searches were conducted from 1945 to March 2016 using the search terms: (Parthenocarp*) AND (genetic mod* OR GM OR genetic* engineer* OR chimeric gene* OR selective breed* OR artificial selection OR hormone) AND (yield OR weight OR Brix). To avoid possible publication bias, patents were included and authors were emailed for relevant reports and unpublished studies (Koricheva, Gurevitch and Mengersen, 2013).

Studies were included that met all the following criteria: (1) They were a pollinator-dependent horticultural crop species; (2) presented an effect of induced parthenocarpy on yield; (3) reported the sample size; (4) reported the mean, and if possible, the standard deviation for each treatment (for independent categorical variables). Methods to induce parthenocarpy were selective breeding or genetic modification (genetic parthenocarpy), or application of growth hormones (artificial parthenocarpy). Each intervention was compared to its own (negative) control. So, selective breeding compared parthenocarpic varieties with non-parthenocarpic varieties (SB), growth hormones compared application with no application (HA), and genetic modification compared modified with non-modified plants (GM). Effectiveness was measured in terms of crop quantity (e.g. weight per plant, or yield) and quality in terms of sugar content (e.g. ° Brix where one-degree Brix is 1 gram of sucrose in 100 grams of nectar).

Authors of the original studies quantified the effect of parthenocarpy (i.e. compared parthenocarpic treatment with non-parthenocarpic control) within different 'test environments' which can be broadly classified into hand pollination (this includes one example of experimental flowers being 'selfed', i.e. fertilised by pollen from the same plant (Molesini *et al.*, 2009)) (hereafter, HP), no pollination, (hereafter, NP), or open pollination (hereafter, OP). In both OP and HP conditions only, pollen from plants of the same genetic material were used. Conditions for which the plants were open pollinated vary between studies, from glasshouses supplemented with *Bombus terrestris* L. colonies to 'open field' conditions. The ecological complexity, i.e. availability of pollinators at these 'open fields' was not provided. These test environments thus have differing background levels of potential pollination and were therefore included

as a fixed effect in the analysis. The reasons for this were twofold: (1) to see if NP conditions resulted in larger effect sizes (due to non-parthenocarpic controls setting no fruit) and likewise smaller effect sizes in OP and HP conditions for the opposite reason (due to non-parthenocarpic controls setting fruit), and (2) to ensure that test environment did not influence treatment effectiveness. For OP and HP conditions to be included in the meta-analysis, authors had to evidence parthenocarpic fruit set through either a much reduced number of seeds or that fruit set occurred in conditions adverse for pollinators (Pandolfini, 2009).

Calculation of Effect Sizes

Within individual studies different plant species, varieties and pure-bred lines may be tested to determine which one has the best parthenocarpic potential for industrial development. Therefore, each genetic line was independent and thus included as separate cases in the dataset. As a result, many studies contributed more than one entry to the dataset. If a study examined multiple years or more than one treatment level of hormone concentration, then the largest sample size, or in cases with equal sample sizes the treatment level with the greatest effect, was selected.

Hedges' *d* was used as a measure of effect size in our meta-analysis. This measure is not affected by unequal sample sizes and includes a correction factor for small sample sizes (Koricheva, Gurevitch and Mengersen, 2013). Hedges' *d* was calculated for each treatment-control pair in the dataset (Table A 2.2), based on the mean, standard deviation and sample size using the 'metafor' R package (Viechtbauer, 2010).

We used bootstrapped analyses to fill in missing standard deviations (22 quantity samples and 4 quality samples), using 1000 resampled data sets

following 'hot deck' imputation, outlined in Koricheva *et al.* (2013). We also include Forest plots showing effect sizes using only complete data (without bootstrapping) in Figures A2.1-2.3.

Meta-analyses

Within a single attempt at inducing parthenocarpy, for example with multiple concentrations of hormones, the concentration which resulted in the greatest effect size (measured by hedges' *d*) was selected. This was done to be representative of how these experimental studies would inform industry, i.e. only the best lines and methods would be put forward for development.

All effect sizes were normalised for their positive skew using a real-solution cube-root transform (following Tukey's ladder of powers). To assess the importance of parthenocarpy-inducing methods on crop quality and quantity, one sample two-tailed t-tests were used. The relative effectiveness of parthenocarpy-inducing methods and the effect of different test environments were investigated with analysis of variance (ANOVA). Possible interactions between these two effects were investigated with generalised linear models, using backward stepwise model selection (Crawley, 2012).

Results

Following a key word search of the literature, 161 studies investigated the effect of parthenocarpy in 33 pollinator-dependent crop species. Of these, 35 did not supply full-text, 8 were not in English, and 78 used a study design unsuitable for inclusion (Table A 2.1~~Table A 2.1~~). The remaining database included 184 effect sizes from 40 studies. Following our selection of the most effective treatments from each experiment (to reflect those which would be taken forward for development) our final sample size was 69 effect sizes (29 for genetic

modification, 31 for hormone application, and 9 for selective breeding) (Table A 2.2). These techniques had been used experimentally and/ or commercially on 18 pollinator-dependent crop species, of which 3 have an 'essential' need, 6 have a 'great' need, 3 have a 'modest' need, and 3 have a 'little' need for insect-mediated pollination (3 pollinator-dependent species were unclassified) (Klein *et al.*, 2007). Tomato was the most commonly studied species (16 studies), followed by aubergine (4 studies) and sweet pepper (3 studies). There was a notable absence of seed and nut crops; this was to be expected given that parthenocarpy causes seedlessness, an undesirable trait in these species. Likewise, an additional 14 pollinator-dependent species showed no evidence of experimental or commercial parthenocarpy in the literature (Table A 2.1~~Table A 2.1~~).

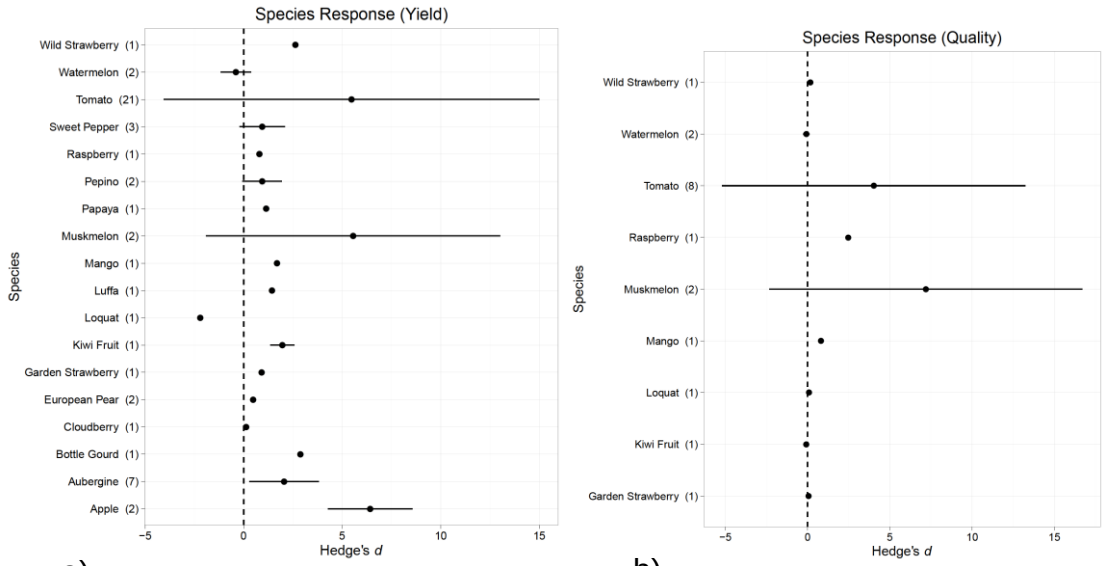


Figure 2.1 Mean effect sizes for all methods combined to induce parthenocarpy (genetic modification, hormone application, and selective breeding) split by crop species (y axis) for (a) fruit quantity (b) fruit quality. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

~~All~~ Across all species methods to induce parthenocarpy significantly increased fruit quantity (T_{50} = 8.41, P < 0.001) (Figure 2.1~~Figure 2.1~~a) and quality (T_{17} = 3.57, P = 0.002) (Figure 2.1~~Figure 2.1~~b). However, there were no significant differences in the effectiveness of genetic modification, selective breeding, or hormone application for increasing fruit quantity (F_{48} = 0.41, P = 0.666) (Figure 2.2~~Figure 2.2~~a) or quality (F_{16} = 0.86, P = 0.367) (Figure 2.2~~Figure 2.2~~b).

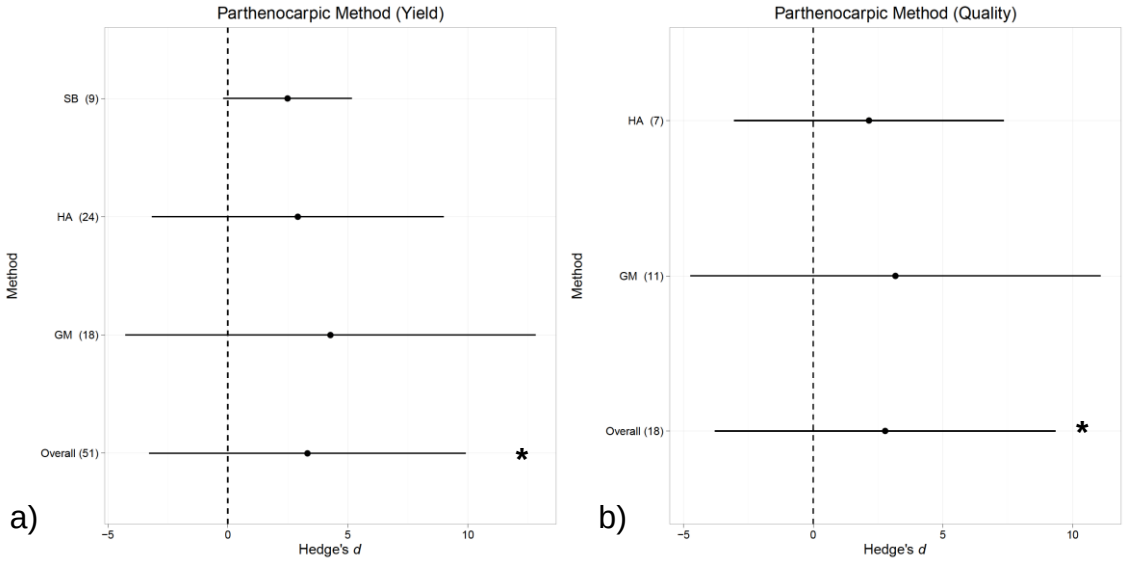
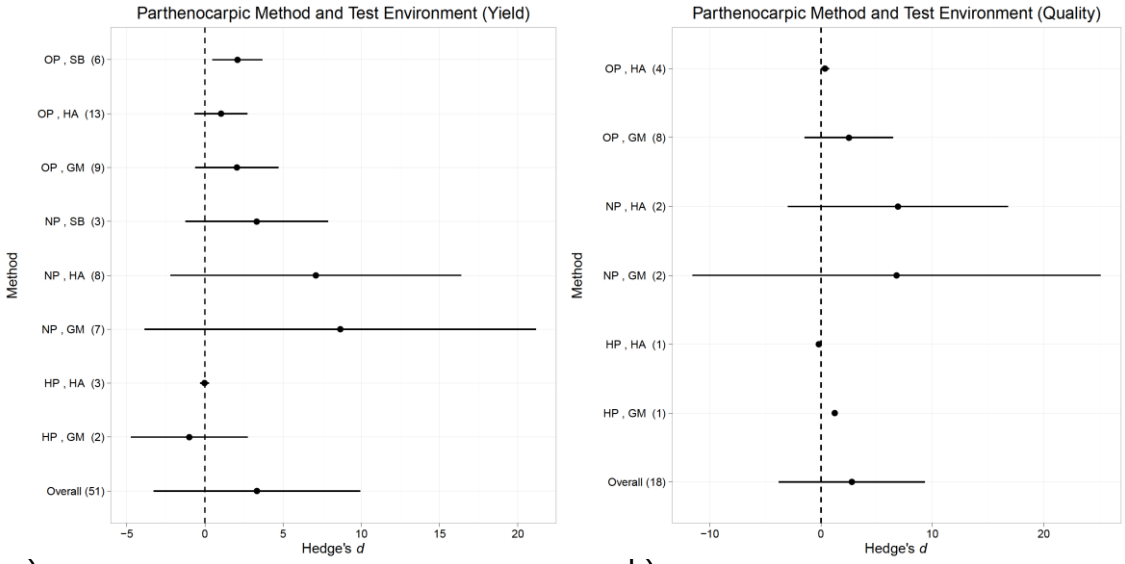


Figure 2.2 Overall mean effect sizes and effect sizes of methods to induce parthenocarpy (genetic modification (GM), hormone application (HA), selective breeding (SB)) (y axis) for (a) fruit quantity and (b) quality for all crop species. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses. Significant one sample two-tailed *t*-tests are indicated with an asterisk (*) (P < 0.05).

Test environment was shown to influence how effective treatments were on fruit quantity (F_{48} = 8.35, P < 0.001), with 'no pollination' environments having the largest effect size (Figure 2.3~~Figure 2.3~~). However, test environment did not influence the effectiveness of parthenocarpy-inducing methods on fruit quality (F_{15} = 0.391, P = 0.683) (Figure 2.3~~Figure 2.3~~b). Notably, there was no interactions between treatments and test environment (F_{43} = 1.63, P = 0.197),

showing that the influence of test environments on treatment effectiveness was not biased against any parthenocarpy-inducing method.



a) b)

Figure 2.3 Overall mean effect sizes and effect sizes of methods to induce parthenocarpy (genetic modification (GM), hormone application (HA), selective breeding (SB)) and test environment (NP, OP, and HP) (y axis) for (a) fruit quantity (b) fruit quality for all crop species. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

Discussion

Artificial and genetic parthenocarpy have proven to be successful at increasing fruit quantity (Figure 2.1~~Figure 2.1~~a), without negatively affecting quality in all crop species studied (Figure 2.1~~Figure 2.1~~b). This is vitally important for commercial acceptance of parthenocarpy as it is only valuable to growers if there are no adverse effects on fruit quality. For example, damaging normal vegetative growth (other than a reduced number of seeds), or a reduction in sugar and nutritional content (Pandolfini, 2009). In this study °Brix was used as a measure of quality as this was the only metric consistently recorded in studies.

The most studied method for inducing parthenocarpy is hormone application, which was shown to positively increase crop quantity and quality (Figure 2.2~~Figure 2.2~~). This method is currently the most widely used by commercial growers, and although usually used prophylactically could be a very good temporary practice for periods of unfavourable environmental conditions.

Selective breeding (creating F1 hybrids) could provide a longer-term solution for inducing parthenocarpy, which despite being investigated in fewer studies, still proved very successful at increasing yield (Figure 2.2~~Figure 2.2~~a). This complements yield trials not included in this meta-analysis (Table A 2.1~~Table A 2.1~~) which have found evidence of genetic parthenocarpy in pollinator-dependent species. For example, 66 per cent of sweet pepper varieties (Honda *et al.* 2012) and 33 per cent of squash varieties examined (Robinson and Reiners 1999) were found to set parthenocarpic fruit. Although an effective method, selective breeding has its limitations. Principally, that crop species can only be crossed with ones that they can sexually reproduce with, and undesirable traits may be inherited alongside desirable ones during crossing. Likewise, selective breeding of varieties is expensive and time consuming, with varieties taking 5-10 years to be released (De Vries, Rabbinge and Groot, 1997). This is because pure lines need to be maintained over many years to ensure their quality, and hybridisation of pure lines often needs to be done by hand. Likewise, seeds grown from F1 hybrids often produce inferior yields to parental crops and consequently growers will need to purchase new F1 seeds each year (Tripp, 1994).

Genetic modification for parthenocarpy could speed up this process by removing the need for back crossing and has been shown to be the most

effective method in this meta-analysis (Figure 2.2~~Figure 2.2~~). This is supported by Donzella *et al.* (2000) who showed genetic modification to be more effective than hormone spraying at increasing yield. The authors concluded that genetic modification enabled a 10 per cent reduction in production costs (less labour needed for the hormonal sprays) and increased profit from improved quality following the genetic modification. Interestingly genetic modification in strawberry and raspberry (Mezzetti *et al.*, 2004), and tomato (García-Hurtado *et al.*, 2012; Medina *et al.*, 2013) has been shown to increase the number of flowers per plant, demonstrating the role that phytohormones also play in fecundity. Therefore, yield per plant may be greater than yield per fruit. Genetic methods could also use alternative methods of genetic engineering such as cisgenesis. This could increase the likelihood of regulatory and consumer acceptance by transferring genes between organisms that could otherwise be conventionally bred (Tester and Langridge, 2010; Telem *et al.*, 2013).

The range of effect sizes observed in this study (Figure 2.2~~Figure 2.2~~) demonstrates the negative effects that unsuccessful parthenocarpy attempts can have on yield, alongside the highly positive effects that successful parthenocarpic treatments can have, for example those shown in tomato and muskmelon (Figure 2.1~~Figure 2.1~~). The variation in the strength of these responses is primarily due to species-specific responses to growth hormones (both applied and genetically modified). For example, if the expression of auxin coding transgenes (in genetically modified) or auxin concentration (from hormone application) is too high, then fruit may appear malformed, particularly in auxin sensitive species (Gorguet *et al.* 2005; Gemici *et al.* 2006). Likewise, relationships between different phytohormones are complex and vary greatly depending on species. This demonstrates the need for continued, multi-

treatment experiments to test the most effective strengths and types of hormones, tailored to individual crop species.

Investigating fruit quality and quantity in different test environments can allow us to assess how useful parthenocarpy could be in the total absence of pollination and fertilisation. In the example of genetically modified aubergine, Acciarri *et al.* (2002) found a 30 to 35 per cent increase in productivity, without any effect on quality under both greenhouse and open field conditions. In both test environments the fruit was always seedless therefore, positively influencing fruit quality and the economic value of production. Larger effect sizes in no pollination conditions (Figure 2.3~~Figure 2.3~~), demonstrate the greater effect that parthenocarpy will have in conditions where fruit set would ordinarily be very low. Consequently in conditions where hand pollination is required for improved fruit set, artificial and genetic parthenocarpy could be a cost effective alternative (Allsopp, de Lange and Veldtman, 2008; Niu, Wang and Li, 2015). Conversely effect sizes tend to be smaller in open and hand pollinated environments where pollen is available (Figure 2.3~~Figure 2.3~~). This is likely to be because in these conditions the non-parthenocarpic controls are successfully pollinated to some extent. However, in all test environments plants may have experienced some pollination deficit (i.e. if plants were selfed, pollinated from just one donor plant, or if experiments were conducted in areas with low pollinator abundance). It is not possible to ascertain the degree of pollination deficit in the HP and OP test environments, and to what extent these limitations represent real world growing conditions. So, these results may over-estimate the effect of parthenocarpy compared to yield resulting from open pollination in an environment where pollinators are not limiting, and natural pollination is thus optimal.

Nonetheless, parthenocarpy could still be useful in open pollination environments, where it can minimise the potential for pollination deficits whilst improving fruit uniformity caused by stochastic poor pollination (Pandolfini, 2009). Therefore, parthenocarpy could be advantageous to all crops, whether or not they are experiencing a pollination deficit. In return, these parthenocarpic crops can continue to provide valuable nectar and pollen resources for our wild and managed bees, and other flower-visiting insects. However, there is no information available as to how the quality and quantity of nectar and pollen varies between parthenocarpic and non-parthenocarpic plants, or how selective breeding for parthenocarpy will affect a plant's nectar and pollen production over time. It is also worth remembering that parthenocarpic fruit set and therefore seedlessness is not always desirable, such as crop species where seeds are the edible part and for creating of seed stock.

Incomplete routes of communication between the plant breeding industry, ecologists, and apiculturists have resulted in a mixed and potentially inaccurate message about the extent of our dependence on pollinators for food production (Ghazoul, 2005; Kleijn *et al.*, 2015; Melathopoulos, Cutler and Tyedmers, 2015). Studies which value the contribution of insects to pollination are based on pollinator dependence, i.e. the extent that a plant depends on pollinators for fruit set. However, this metric assumes that dependence is constant within a single crop (Klein *et al.*, 2007; Gallai *et al.*, 2009). In reality pollinator dependence is strongly dependent on variety, the spatial and temporal context of the surrounding landscape, and the responses of farmers, consumers and technological innovation to pollinator decline. Therefore, we highlight that there may be over-estimation of pollinator dependence if studies overlook research and development currently underway to reduce the need for pollination. We

found evidence for studies inducing parthenocarpy in four out of 13 of the global crops for which pollination is considered essential (according to Klein *et al.* 2007); and 13 out of 30 of the crops for which the need for pollination is considered great. This indicates that research into reducing *demand* for pollination has occurred in 40% of the crops for which ecologists are currently primarily only advocating an increase in *supply* of pollinators as the solution to improving crop yields and quality (Garibaldi *et al.*, 2011; Carvalheiro *et al.*, 2013). Indeed, there are three crop species in the top twenty crops for global production (Mt/yr in Klein *et al.* 2007) which benefit from insect pollination and appear in this meta-analysis of parthenocarpy studies (tomato #12; watermelon #15; apple #19). It is not surprising that, if a crop is showing a yield deficit, then different routes are explored to solve the problem; but it is surprising that evidence of the effectiveness of different approaches is not brought together more comprehensively to build an accurate picture for a crop. Single successes presented in this meta-analysis could lead to profound changes in production of certain crops, for example nearly all bananas on the global market are of the Cavendish variety, selectively bred to be parthenocarpic.

Data are not currently available to assess variety choice by farmers, or the level of parthenocarpy in the varieties that they choose. The results of this meta-analysis support the conclusions of Klein *et al.* (2007) and Melathopoulos *et al.* (2015) that to get a more complete picture, varietal information is required – both in terms of pollinator dependence, but also in terms of choices that farmers are making.

Conclusions

Parthenocarpy may be able to reduce the need for pollinators in many horticultural crops but should not be used as a panacea for agricultural success. Biodiversity decline in agricultural landscapes is often an indicator of poor ecosystem health, which can also cause poor fruit set. Thus, agricultural growers should carefully consider causes of poor fruit set and ideally use parthenocarpic species (which can still provide an important nectar and pollen source for pollinator species) in addition to other environmentally considerate practices. Likewise, parthenocarpy could further the pollinator crisis by removing the imperative for conserving pollinators as our 'dependence' on them is reduced (Brown *et al.* 2016). This could affect pollination of non-parthenocarpic pollinator-dependent crops as well as wild plants. Ultimately, widespread implementation of these practices will be limited to countries that have access to and can afford skilled personnel and equipment. Thus, free communication of resources and capabilities from developers to users is essential for the benefits of parthenocarpy to reach the areas of the world that are most in need of its benefits.

This study shows that genetic and artificial parthenocarpy has a great potential to improve fruit quantity, without affecting quality in a range of horticultural crops. Potentially the most promising method for inducing parthenocarpy is genetic modification; the most effective for increasing fruit quality and quantity, whilst being the quickest to implement. However, whilst acceptance for genetic modification, particularly in Europe, remains equivocal, selective breeding may be a more attainable way for achieving genetic parthenocarpy. This method is also relatively cost-effective for many horticultural growers already growing hybrid varieties. Although currently a popular choice, hormone application

remains an expensive and un-sustainable option for many horticultural growers. Nonetheless, any additional costs for agricultural growers associated with implementing genetic and artificial parthenocarpy could be offset by increasing the quality and quantity of crops. Unfortunately, no studies have directly compared the cost of parthenocarpy to traditional methods of supplemented pollination, such as introduced honeybee hives and hand pollination. Climate change could also increase pressure to develop parthenocarpic crop species as changes in pollinator distributions or declines in their populations are likely to be detrimental to food production (Kerr *et al.*, 2015). Thus, parthenocarpic crop plants could allow producers to extend their growing seasons in otherwise adverse climatic and environmental conditions, furthering their economic advantage, increasing agricultural resilience, and improving food security.

3. Courgette production: pollination demand, supply, and value

Systematically reviewing the literature for Chapter 2 showed how little information is available on pollinator dependence within, and between crop species. Since there is no data available on field-grown Tosca, the courgette variety used in this study, I conducted pollination experiments to estimate pollinator dependency and to see if this variety was experiencing a pollination deficit at field sites in Cornwall. Since pollinator dependency and pollination deficit combine to directly influence yield I used economic valuations to clearly demonstrate to growers the importance of conserving pollination services for courgette production in the UK.

Plate 6 *B. terrestris* covered in courgette pollen after visiting a staminate flower for nectar. The empirical work for this chapter was the first time I appreciated just how abundant and effective a pollinator *B. terrestris* is in courgette. This concept is further examined in Chapters 4 and 5. Photograph by Daphne Wong.

Abstract

Courgette (*Cucurbita pepo* L.) production in the UK is estimated to be worth £6.7 million. However, little is known about this crop's requirement for insect-mediated pollination (pollinator dependence) and if pollinator populations in a landscape can fulfil its pollination needs (pollination deficit). Consequently, pollination experiments were conducted over two years to explore pollinator dependence and pollination deficit in field grown courgette in the UK. Results showed that pollination increased yield by 39% and there was no evidence of pollination limitation on crop yield. This was evidenced by a surprisingly low pollination deficit (of just 3%) and no statistical difference in yield (length grown, circumference, and weight) between open- and hand-pollinated crops. Nonetheless, the high economic value of courgettes means that reducing even the small pollination deficit could still increase profit by ~£166/ha. Interestingly, 56% of fruit was able to reach marketable size and shape without any pollination. Understanding a crop's requirement for pollinators can aid growers in their decision making about what varieties and sites should be used. In doing so they may increase their agricultural resilience and further their economic advantage.

Introduction

As agriculture intensifies and habitat conversion to farmland continues, crop producers are frequently relying on managed pollinator species to fulfil their pollination needs (Mader, Spivak and Evans, 2010). Increasing the abundance of species such as *Apis mellifera* L. can interrupt the damaging cycle of lower yields from a reduced diversity and abundance of wild pollinators, often caused by losses in (semi-) natural habitat (Garibaldi *et al.*, 2011). This is a common practice for growers of Cucurbitaceae (cucurbits or gourds) (Free, 1993), a large and genetically diverse plant family which are thought to have an 'essential' requirement for insect-mediated pollination (Klein *et al.*, 2007). In these cucurbit-growing areas an increase in the supply of pollinators is advocated in almost all situations, regardless of surrounding landscape (Nerson, 2007). However, there is concern that pollination services provided by managed and wild bees are still not enough to fulfil requirements for crop production (Schulp, Lautenbach and Verburg, 2014).

Consequently, many studies have attempted to quantify pollination deficit: the difference between current and optimum levels of pollination. Experimentally increasing the abundance of pollinators has been shown to increase yield of summer squash (Artz & Nault 2011; Nerson 2009), melon (Kouonon *et al.* 2009; Nerson 2009), and cucumber (Nerson 2009). Likewise, areas with a high diversity of bee species may also benefit from increased yield, as evidenced with pumpkin (Hoehn *et al.* 2008). This positive relationship between pollinator visitation and yield means that fruit set is directly dependent on pollinators and the ecosystems which support their populations. Therefore, results are highly dependent on the spatial and temporal context of the landscape surrounding each crop field.

Whilst these positive relationships demonstrate how a crop can benefit from insect pollination, they do not quantify a crop's requirement for insect-mediated pollination or 'pollinator dependence'. This is quantified by comparing fruit set from open- or hand-pollinated flowers to flowers which have had pollinators excluded. Excluding pollinators from some cucurbits has shown that fruit set is unable to occur (Hoehn *et al.*, 2008) and that increased pollen loads can make fruit grow faster and larger (Stephenson, Devlin and Horton, 1988; Artz and Nault, 2011). However, the dependence of a crop species on pollinators is likely to vary between varieties (Knapp, Bartlett & Osborne 2016, Chapter 2). For example, 22 out of 33 summer squash varieties have been shown to set fruit without pollination (Robinson and Reiners, 1999). Likewise, fruit set without pollination has also been observed in cucumber (Kushnereva, 2008), watermelon (Sedgley, Newbury and Possingham, 1977), and additional varieties of summer squash (Martínez *et al.* 2013; Martínez *et al.* 2014; Kurtar 2003). This type of fruit set, without pollination and therefore fertilisation, is parthenocarpy. As evidenced by these accounts of cucurbit growing, understanding a crop's requirement for pollination and, in turn, how pollinators vary spatially and temporally in the landscape is essential to design and deliver optimum crop management. The economic value of pollination can be included in cost-benefit analyses to inform decision making at a farm and policy level (Hanley *et al.*, 2014). This is because valuation based on a crop's dependence for pollination will show the detrimental impact that a decline in pollinator populations may have, and valuation based on the pollination deficit will show the potential that increasing pollinator populations may have. Consequently, quantifying the economics of pollination is a fundamental way for growers to understand the implications that changes in pollinator populations may have on

their yield and economic return. Despite the economic importance of many cucurbit species and their 'dependence' on pollination, no studies have calculated the economic value of pollination to cucurbit crops. In other high value crops such as apple, economic valuations have shown that maximising pollination could increase UK output by £5.7 million per year (M. P. D. Garratt *et al.*, 2013).

In the UK, the nutritional value of cucurbits has increased their popularity and therefore, supermarket demand. To receive maximum profit from consumers, each supermarket has their own quality specifications which they require growers to achieve. Consequently, growers strive to produce perfectly formed fruit to ensure an adequate return for their efforts. This study focuses on the pollination dynamics of field-grown courgettes (*Cucurbita pepo*) as a model species for cucurbit crops, which, although grown over a relatively small area in the UK (mostly in Cornwall, Cambridgeshire, Worcestershire, and Sussex), are a high value crop (~£8,000 per Ha). Therefore, to understand whether the dynamics of pollination are affecting yield quality or quantity and to improve guidance to growers for obtaining productive and sustainable yields, we ask: (1) Does pollination influence growth rate, quality and quantity of fruits? (2) Are courgettes experiencing a pollination deficit and does this increase with distance into a field? And (3) what is the estimated economic value of pollinators and their potential profitability to courgette production in the UK? These studies use the popular courgette variety 'Tosca', a high yielding, compact variety which is notably tolerant to powdery mildew, making it a popular choice for commercial production (P.E. Simmons and Son, personal communication 29th June 2016). Despite the potential for parthenocarpy,

selectively-bred parthenocarpic courgette varieties are not currently grown at a commercial scale in the United Kingdom.

Methods

Study sites

In 2015 and 2016, the pollination conditions of Courgette (var. 'Tosca') were manipulated in seven fields across Cornwall, UK. Tosca is a popular courgette variety in the UK, representing 37.9% of the market share (P.E. Simmons and Son, personal communication 1st April 2017). Courgettes were grown in outdoor (opposed to protected) conditions at a density of 13,585 plants per hectare. Each field (average field size of 5.2 ± 1.3 ha (*SE*)) was situated more than 2km apart to ensure independent pollinator communities between fields (Vaissière, 2010) and was conventionally managed with minimum chemical input other than fungicidal sprays (P.E. Simmons and Son, personal communication 29th June 2016). In 2015, 180 flowers were manipulated at two fields and in 2016, 300 flowers at five fields, totalling 480 flowers over the course of the study (Figure 3.1).

Pollination treatments

As courgette is monoecious, each pistillate flower was assigned to one of the following treatments: hand pollination (n = 60), open pollination (n = 60), or no pollination (n = 60) in 2015 and hand pollination (n = 100), open pollination (n = 100), or no pollination (n = 100) in 2016. Hand pollinated flowers were treated on the first day of anthesis around 08:00 h with pollen from a staminate donor flower (from a neighbouring plant) using a paint brush. Open pollinated flowers were left to be pollinated naturally by insects visiting the fields. The no pollination treatment was initiated the day before expected anthesis by securing

PVC mesh bags with wire ties to pistillate flowers. Bags had a mesh size of 0.2mm, designed to be permeable to wind and rain yet exclude any pollinators. To the best of our knowledge no commercially reared *B. terrestris* L. or *A. mellifera* colonies had been introduced within a 1 km radius of each farm. The level of pollinator dependence (the difference between open- or hand- and no pollination) can be interpreted as courgette's 'demand' for pollen, whilst the pollination deficit (the difference between hand- and open-pollinated crops) indicates the 'supply' of pollen in the landscape relative to maximal pollination.

All experimental flowers were individually identified with marker pen written on pieces of flagging tape, tied to the base of each fruit. To avoid the confounding effect of a plant investing in additional fruits from un-monitored pollination events, only one fruit per plant was studied (Stephenson, Devlin and Horton, 1988; Avila-Sakar, Krupnick and Stephenson, 2001).

Quantity and quality measures

In 2015 and 2016 fruits were harvested 10 days post-anthesis, weighed on scales, measured using a tape measure (length and circumference (circumference only in 2015)), and their sugar content (°Brix) recorded (only in 2016). °Brix is considered to be a simple and objective measure which can be used by growers to assess fruit quality, since sweetness is appreciated by consumers (Kleinhenz and Bumgarner, 2013). °Brix was measured on a hand-held refractometer (Bellingham-Stanley, range 0 - 50%) by taking a homogenised value from three 1cm^2 pieces of fruit (middle and either end).

Experimental fruits were classed as 'aborted' if they did not meet minimum commercial standards (Ellis Luckhurst, personal communication 24th June 2015), i.e. they were less than 14 cm long, 30 mm wide (at the mid-point), and

over 5° in curvature, or showed any obvious signs of bacterial damage, such as blossom end rot. Therefore, fruit set (the ratio of marketable fruit compared to the total number of marked flowers per treatment) is also a measure of fruit quality. As fruit set was measured over 10 days courgettes were generally larger than commercial standards. Since these experiments were conducted at a commercial farm some fruits were accidentally removed by pickers. Consequently, final sample sizes were less than the number initiated and are not completely balanced between treatments (hand pollination n = 151, open pollination n = 157, no pollination n = 153).

Effect of pollination over time

In 2015, 180 of the experimental pistillate flowers were measured at two fields (hand pollination (n=60), open pollination (n=60) and no pollination (n=60)) (Figure 3.1). Fruit length was measured daily from the first day of anthesis to 10 days post-anthesis to explore the effect of pollination treatment on fruit length over time. All pollination treatments were conducted simultaneously within each field to minimise environmental variation between treatments.

Pollination with distance into a crop

In 2016, a total of 100 experimental flowers were left to be pollinated naturally in five different fields at 0 m (n = 50) and 50 m (n = 50) into the crop from the field edge (10 flowers per field and location into the crop) (Figure 3.1). In each field, the edge of the crop was a hedgerow. Therefore, 0 m into the crop was closer to semi-natural habitat than 50 m in the crop. To observe bee visitation, three flowers (staminate and pistillate) (on the first day of anthesis) were randomly selected at each of these locations. This method was used (rather than sampling a unit area) because it was the best way of observing multiple flowers

simultaneously. Most pollinator species were *Apis mellifera* and *Bombus* species, so only these were identified to species level. *Bombus terrestris* and bees belonging to the *Bombus lucorum* complex were combined in a single group due to difficulties in reliably distinguishing workers in the field (Murray *et al.*, 2008). Bee visitors were recorded over two 15-minute periods, at each field and location within the crop (0 m and 50 m from the edge), totalling 4 observational periods per field. Pollinator visitation rate was calculated as the number of visits per minute per flower summed across the two surveys for each of the two distances from the edge of the crop. All observations were done in sunny or mild weather conditions (>15 °C) with at most, light wind, between 09:00 and 11:00 h (when flowers were open).

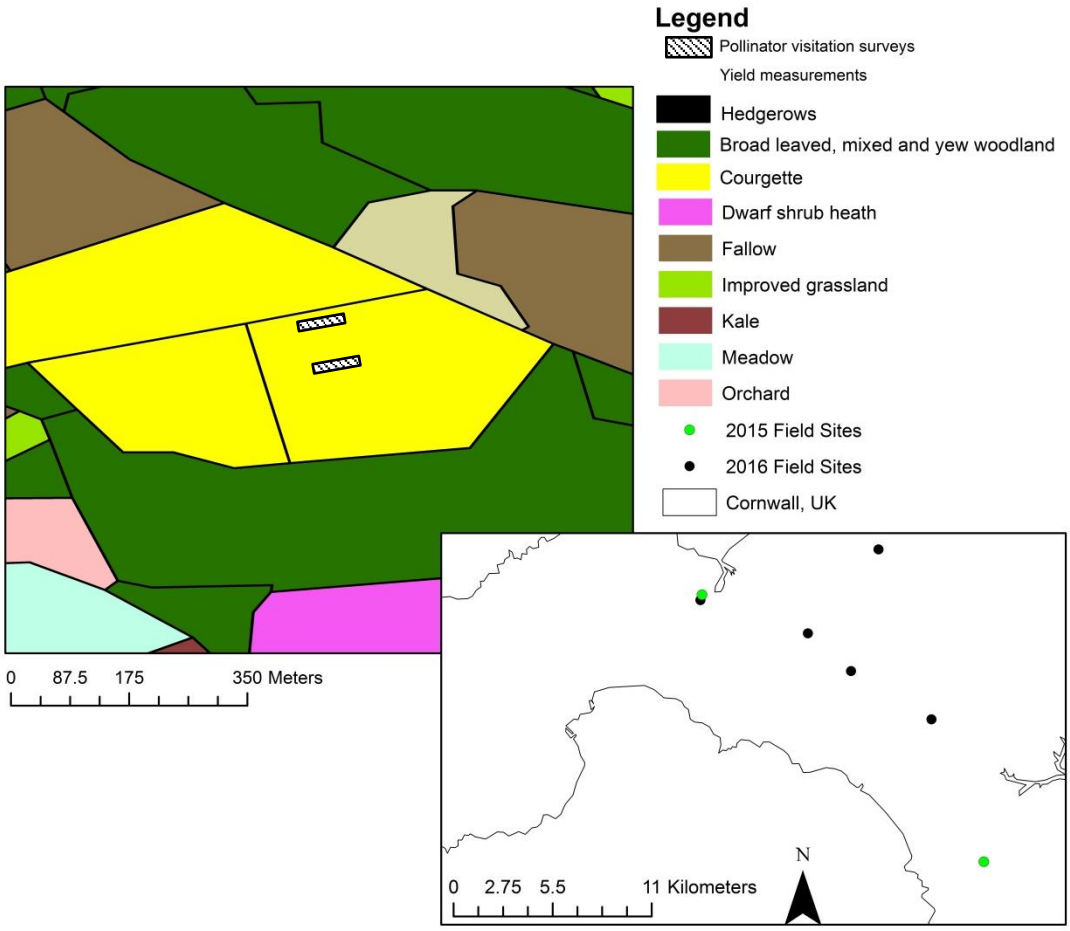


Figure 3.1 One field site showing the locations of pollinator visitation surveys and yield measurements at 0 m and 50 m from the edge of the crop. In 2015

only, yield measurements at 0 m from the edge of the crop were conducted. The location of all field sites (two sites in 2015 and five sites in 2016) are also shown in the context of Cornwall, UK.

Economic value of pollination

It is often assumed that a loss of pollinators will decrease the value of horticultural crops however; yield is also dependent on variety, management practices, and environmental conditions (Bos *et al.*, 2007; Boreux *et al.*, 2013; Klein *et al.*, 2014; Motzke *et al.*, 2015). As these inputs improve, fruit quantity (fruits produced per plant over a season) and fruit quality (size and shape) will increase, improving the grower's economic advantage. Based on Melathopoulos *et al.*, (2015) the economic value of these combined factors (under open pollination conditions) can be broadly estimated as:

$$EV = P \times Q \quad (1)$$

Where *EV* (£/Ha or £ for UK) is the total economic value/ unit area, *P* is the price (£/kg), and *Q* is the quantity of crop grown (Kg/Ha or Kg in UK). To estimate the economic value of courgettes for the UK, and the proportion which depends on insect pollination, we have used national statistics and local data. *P* was calculated as the average weekly price (£/kg) of all courgette varieties (data were unavailable for individual varieties) from June to September 2016 (DEFRA, 2016). *Q* was the average yield (kg/ha) of one courgette variety, Tosca, at the 2015 study site in Cornwall (P.E. Simmons and Son, personal communication 29th June 2016).

Using the pollination manipulations in this study, a coefficient of pollinator dependency (*D*) can be calculated as the fruit set as a result of open pollination

(f_p) compared to pollinator exclusion (f_{pe}). *D* relates to pollinator dependency in particular conditions, whereas *Dmax* is the maximum dependency of a crop on pollinators. *Dmax* is calculated as the fruit set as a result of hand pollination (f_{pmax}) compared to pollinator exclusion (f_{pe}). These can be used to determine the extent to which fruit set would increase or decrease if pollination was improved or removed.

$$D(or\ D_{max}) = 1 - \frac{f_{pe}}{f_p\ (or\ f_{pmax})}$$

(2)

To calculate the economic value of pollination (*IPEV*), i.e. the proportion of the crop's value that would be lost if all pollinators were removed, the total value of the crop (per hectare) is multiplied by *D*.

$$IPEV = EV \times D$$

(3)

On the other hand, if pollination was maximised (equivalent to hand pollination) then the maximum economic value (*MaxEV*) of courgettes would be:

$$MaxEV = EV \times D_{max}$$

(4)

Subtracting *IPEV* from *MaxEV* reveals the pollination deficit (*PDef*) at a particular location. This is the potential profitability that pollinators could provide under maximal pollination conditions.

$$PDef = MaxEV - IPEV$$

(5)

[For further explanation of these equations, see Melathopoulos *et al.* (2015)].

EV, *IPEV*, *MaxEV* and *PDef* were all calculated for courgettes and then multiplied by the total area of courgette production (for all varieties) in the UK (British Growers Association, personal communication 22nd September 2016)) to calculate values for UK production. Owing to a lack of data (in this study and the wider literature) on pollinator dependence and the area of different courgette varieties in the UK, figures are only based on one courgette variety (Tosca) for *D* and all varieties for *P* and *Q*.

Statistical analysis

All analyses were performed in the R package lme4 (Bates *et al.*, 2015). Error distributions were Gaussian unless otherwise stated and residual plots were used to check for normality and heteroscedasticity. *Post hoc* Tukey tests were calculated using the multcomp package (Hothorn, Bretz and Westfall, 2008).

2.8.1 Pollination treatment

The effect of pollination treatment (fixed effect) on fruit growth (length 10 days after anthesis) (2015 and 2016 data combined), weight (2015 and 2016 data combined), circumference (2015 data only) and °Brix (2016 data only) was tested with field specified as a random effect.

Fruit set (the ratio of marketable fruit compared to the total number of marked flowers per treatment) was modelled using a GLM with a binomial error distribution with field and pollination treatment as fixed effects.

2.8.2. Pollination with distance into the crop

Fruit set (with a binomial error distribution), fruit growth (length after 10 days), weight, and °Brix under open pollination conditions were assessed in relation to distance from the edge of the crop, pollinator visitation rate (visits per minute

per flower, summed across the two surveys for each of the two distances from the edge of the crop) and their interaction as fixed effects and field was specified as a random effect. Pollinator visitation rate was assessed in relation to distance from the edge of the crop with field specified as a random effect.

Results

Pollination deficit and pollinator dependence

Fruit set of Tosca (in 2015 and 2016) significantly increased with hand- and open-pollination compared to no-pollination conditions however, there was no significant difference between hand- and open-pollination (Table 3.1~~Table 3.1~~). Overall fruit set was 98% for hand pollinated flowers, 95% for open pollinated flowers and 56% under no-pollination conditions (Table 3.1~~Table 3.1~~). Over half of the experimental flowers subjected to the no pollination treatment were able to set fruit to marketable size and weight (Table 3.1~~Table 3.1~~). ~~However~~However, fruit length, weight and circumference (not °Brix) for non-pollinated flowers were significantly decreased compared to hand- and open-pollinated flowers (Table 3.1~~Table 3.1~~).

Table 3.1 Results from the LMMs and GLM on the effect of pollination treatment (hand pollination, open pollination and no pollination) on field grown courgette quality and quantity measures (mean ± standard error). N = the number of fruits analysed. *Post hoc* Tukey tests used to test for differences in pollination treatment are shown.

				Tukey *post hoc* tests		
Measure	**Hand pollinated (mean ± *SE* (n))**	**Open pollinated (mean ± *SE* (n))**	**Pollinator exclusion (mean ± *SE* (n))**	**Contrast estimate ± *SE***	**Test statistic (z-value)**	***P*-value**
Fruit set (%)	98 ± 2.2 (151)	95 ± 2.9 (157)	56 ± 10.9 (153)	HP - NP: 2.71 ± 0.82 OP - NP: 2.35 ± 0.77 HP - OP: 0.35 ± 0.84	3.31 3.07 0.42	**0.003** **0.006** 0.91
Fruit growth (length in cm after 10 days)	22.8 ± 0.5 (148)	22.0 ± 0.5 (149)	16.5 ± 0.8 (86)	HP - NP: 7.16 ± 0.68 OP - NP: 6.26 ± 0.67 HP - OP: 0.9 ± 0.57	10.56 9.26 1.56	**<0.0001** **<0.0001** 0.26
Fruit weight (g)	829.9 ± 35.1 (148)	768.3 ± 33.2 (149)	520.1 ± 41.6 (86)	HP - NP: 362.6 ± 42.38 OP - NP:298.16 ± 42.27 HP - OP: 64.44 ± 35.8	8.56 7.05 1.8	**<0.0001** **<0.0001** 0.17
Fruit circumference (cm)	17.4 ± 0.5 (60)	18.5 ± 0.7 (60)	15.0 ± 0.5 (60)	HP - NP: 7.43 ± 0.75 OP - NP: 6.73 ± 0.74 HP - OP:	9.96 0.94 9.09	**<0.0001** **<0.0001** 0.62

				0.7 ± 0.74		
Brix	3.8 ± 0.04 (88)	3.8 ± 0.04 (89)	3.8 ± 0.08 (54)	HP - NP: 0.002 ± 0.08 OP - NP: 0.07 ± 0.07 HP - OP: 0.06 ± 0.08	0.03 1.03 0.86	1.0 0.67 0.56

Effect of pollination over time

Despite fruit length remaining similar for the first four days (just before fruits achieve a minimum marketable weight), non-pollinated fruits did not grow as long in length as open- and hand-pollinated fruits (Figure 3.2~~Figure 3.2~~).

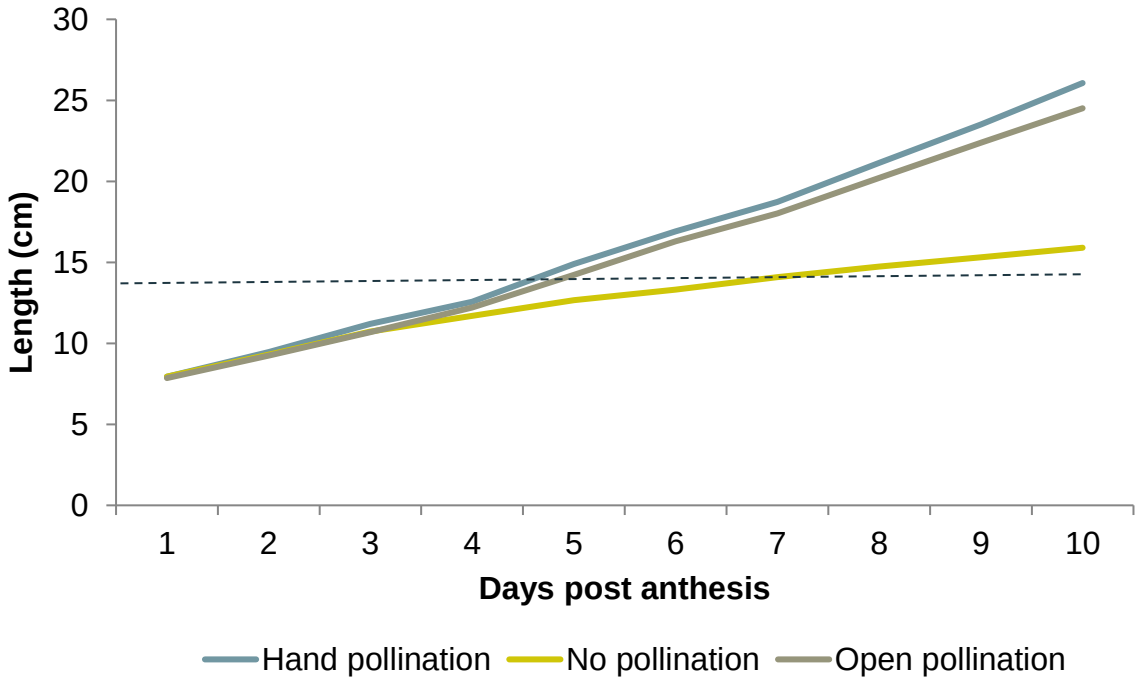


Figure 3.2 Average daily length (y axis) of field grown courgettes subject to pollination treatments (hand pollination, open pollination and no pollination) over 10 days (x axis). The dashed line shows the minimum length required for commercial courgettes.

Pollination with distance into a crop

Distance from the edge of the crop had no effect on percentage fruit set, fruit growth, weight and °Brix of open-pollinated plants (Table 3.2~~Table 3.2~~). Likewise, pollinator visitation rate (contrast estimate -4.68 ± 2.899 *SE*, *Z* = -1.587, *P* = 0.11) and the interaction between distance from the edge of the crop and pollinator visitation rate (contrast estimate 1.45 ± 4.33 *SE*, *Z* = -0.336, *P* = 0.74) did not influence fruit set. Overall, there was no change in pollinator visitation rate with distance from the edge of the crop (contrast estimate 0.04 ±

0.05 *SE*, *T* = 0.72, *P* = 0.47). However, *Bombus* spp. were more abundant at the edge of the crop, unlike *Apis mellifera* which were more abundant within the crop (Figure 3.3~~Figure 3.3~~).

Table 3.2 Results from the GLMMs and LMMs on the effect of distance from the crop edge on field grown courgette quality and quantity measures (mean ± standard error). N = the number of fruits analysed.

Measure	**0 m from the crop edge (mean ± *SE* (n))**	**50 m from the crop edge (mean ± *SE* (n))**	**Contrast estimate ± *SE***	**Test statistic**	***P*-value**
Fruit set (%)	92 ± 5.8 (5)	97.8 ± 2.2 (5)	0m - 50m: 0.95 ± 1.64	Z = 0.576	0.56
Fruit growth (length in cm after 10 days)	26.3 ± 0.7 (45)	24.3 ± 0.9 (44)	0m - 50m: -2.65 ± 2.39	T = 1.106	0.27
Fruit weight (g)	1009.3 ± 53.3 (45)	923.1 ± 61.7 (44)	0m - 50m: -147.51 ± 167.14	T = 0.883	0.38
Brix	3.8 ± 0.1 (45)	3.9 ± 0.1 (44)	0m - 50m: -0.12 ± 0.20	T = 0.615	0.54

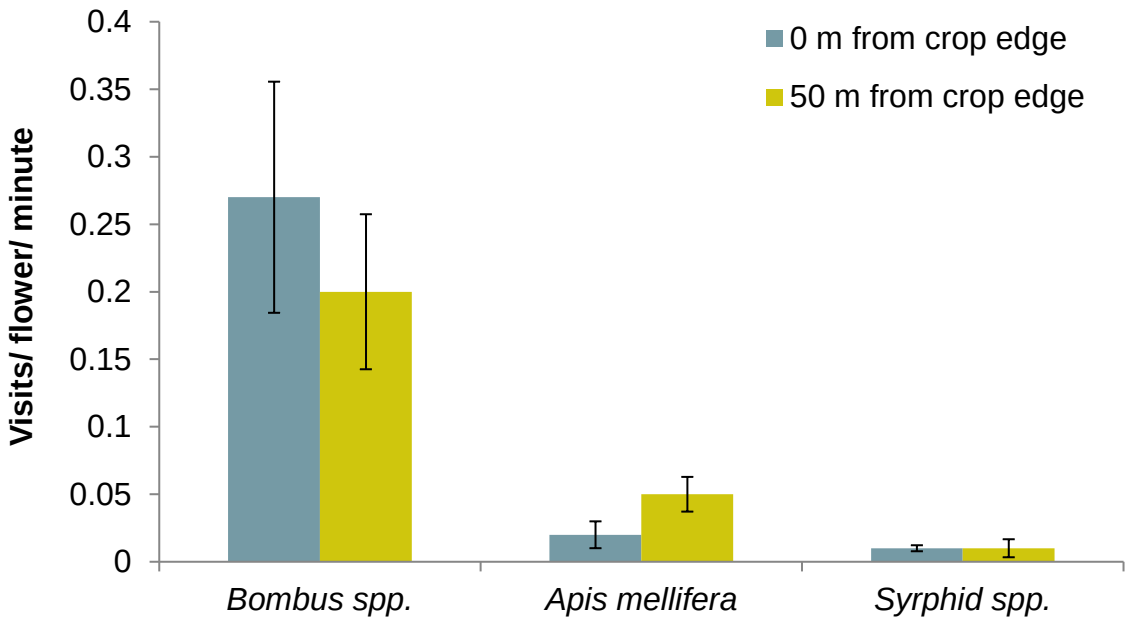


Figure 3.3 Flower visitation rate for *Bombus* spp. (*B. terrestris/ lucorum, B. pascuorum* and *B. hortorum* combined), *Apis mellifera* and *Syrphid* spp at 0 m and 50 m from the edge of courgette fields in 2016. Mean ± *SE* (n = 10). There was no change in pollinator visitation rate with distance from the edge of the crop (contrast estimate 0.04 ± 0.05 *SE*, $T = 0.72$, $P = 0.47$).

Economic value of pollinators

Courgettes are grown over 808 ha in the UK, which is not a large area compared to other crops, but each hectare of courgettes is worth over £8,000 to the grower in market value (Table 3.3~~Table 3.3~~). The current economic value (EV) of courgettes in the UK is therefore estimated to be £6,694,632. Our pollination experiments demonstrate that the crops studied had a *D* of 0.41 i.e. 41% of fruit set was dependent on natural pollination (f_p compared to f_{pe}). This means that, if all UK crops are pollinated as well as they are in Cornwall, then pollinators contribute £2,744,735 to the total economic value of courgettes in the UK (*IPEV*). The maximum dependency on pollinators under maximal pollination conditions (f_{pe} compared to f_{pmax}) was 0.43. Therefore, if the pollination deficit observed from our pollination experiments (although not

significantly different from open pollination) is assumed to be similar across the UK, then there is scope to improve crop pollination by just 3% which will increase the value of courgettes in the UK by £134,086 (Table 3.3~~Table 3.3~~).

Table 3.3 Calculation of the economic value of pollinators to courgette production at a hectare and national scale. *P* was 0.43 £/ha (DEFRA 2016). Total area of UK courgette production is 807.75 ha (British Growers Association, personal communication 22nd September 2016). *D* was 0.41 and *Dmax* 0.43 calculated from experimental results in Table 3.1~~Table 3.1~~**.**

	Economic value (£)	
	Per ha	UK Value
Quantity *Q* (Kg)	19,274	-
Economic value *EV* (£)	8,288	6,694,632
Total economic value of insect pollination *IPEV* (£)	3,398	2,744,735
Maximum economic value of pollination service *MaxEV* (£)	3,564	2,878,821
Value of pollination deficit *PDef* (£)	166	134,086

Discussion

The importance of pollinators to courgettes is demonstrated through a significant reduction in fruit size and weight under no pollination conditions. Consequently, percentage fruit set, the size and weight, but not sugar content, of courgettes were significantly increased with pollination. As all flowers within a field experienced the same environmental conditions, the observed reduction in fruit set (for non-pollinated and open pollinated flowers) was due to the absence of pollen. The relatively high fruit set of hand pollinated flowers (98%) suggests that resources (such as nutrient and water availability) were unlikely to be limiting courgette growth and fruit set and demonstrates the quality and quantity of courgettes under optimal pollination conditions. Unfortunately, it was

impossible to identify any differences in pollinator dependence between courgette varieties as data from this study are only available for one courgette variety.

Nonetheless, it is of industrial and ecological interest that 56% of non-pollinated flowers were still able to reach marketable size and shape without any pollination at all. This is due to the natural parthenocarpic tendency of courgettes, previously observed in Tosca (Martínez *et al.*, 2013) and other varieties (Robinson and Reiners, 1999). However, Martínez *et al.*, (2013) concluded that Tosca was not truly parthenocarpic as fruits consistently showed a burst in ethylene around three days after anthesis which is thought to cause early fruit abortion in non-pollinated flowers. This may explain the slower growth rate around three days post anthesis (Figure 3.2~~Figure 3.2~~) and reduced fruit set in non-pollinated flowers (Table 3.1~~Table 3.1~~). The effect of parthenocarpy appeared to have no effect of sugar content in courgettes, unlike observations in melon (Hayata *et al.*, 2000; Shin, Park and Kim, 2007).

The level of open pollination at the study sites was very high, evidenced by no statistical difference in yield (length grown, circumference, and weight) of open- and hand-pollinated crops, and an average pollination deficit of just 3%. Similarly, distance from the edge of the crop had no effect on yield (length grown, weight and °Brix) of open pollinated courgettes, likely related to no difference in bee visitation at 0 m and 50 m from the crop edge (Figure 3.3~~Figure 3.3~~). This may be because 50 m from the crop edge is not far enough from natural or semi-natural habitat (such as hedgerows) to detect differences in pollinators. This is to be expected given that even ‘door step foragers’ such as *B. muscorum*, *B. pascuorum* and *B. lapidarius* are known to forage at

distances greater than this (Walther-Hellwig and Frankl, 2000; Darvill, Knight and Goulson, 2004; Knight *et al.*, 2005). Distance from the edge of the crop is unlikely to be a problem for the majority of cucurbit fields in Cornwall, where the average distance to the centre of a field is around 100 m (average field size of 5.2 ± 1.3 ha (*SE*)) but could be more likely for cucurbit fields in Cambridgeshire where the average distance to the centre of a crop is around 200 m (average field size of 16.5 ± 3.1 ha). Likewise, spatial and temporal variation in the landscape surrounding each field may influence the level of open pollination. For example, other studies have demonstrated that sites situated nearer to natural and semi-natural habitat are more likely to have a greater species richness of pollinators and higher pollination rate (Kremen *et al.*, 2004; Morandin and Winston, 2006; Garibaldi *et al.*, 2011). Studies have also shown that larger fields (particularly towards the centre) are more likely to have lower species richness and reduced pollination rate (Artz, Hsu and Nault, 2011; Garibaldi *et al.*, 2016).

High levels of open pollination observed in this study are attributed to a high abundance, but not diversity, of pollinators as *B. terrestris/ B. lucorum, B. hortorum, B. pascuorum* and *Apis mellifera* were the only bee species recorded (Figure 3.3~~Figure 3.3~~, see also Figure 4.2~~Figure 4.2~~ (Chapter 4) and Figure 5.4 (Chapter 5). This highlights that only a few abundant species, rather than high species richness (contrary to a previous study on pumpkins (Hoehn *et al.*, 2008) and watermelons (Kremen, Williams and Thorp, 2002)), can deliver pollination services to a whole crop (Kleijn *et al.*, 2015; Winfree *et al.*, 2015). However, any loss of these functionally important species could greatly reduce pollination services (Larsen, Williams and Kremen, 2005). Fortunately, these species are generally widespread, resilient to agricultural expansion and can be encouraged

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through simple conservation measures (Kleijn *et al.*, 2015). Observations of pollinator visitation and yield in this study also show that the pollination requirements of courgette can be fulfilled without squash and gourd bees (belonging to the genera *Peponapis* and *Xenoglossa*) which have previously been regarded as the most important pollinators of *Cucurbita* crops in North America (Hurd, Linsley and Michelbacher, 1974).

Since courgette yield is dependent on pollination (D = 0.41) the total economic value of insect pollination to courgettes is estimated to be worth approximately £3,398/ha and is consequently a significant proportion of the total economic value of courgettes (Table 3.3~~Table 3.3~~). Due to high levels of open pollination observed in Cornwall, pollination deficit was estimated to be just 3%. Nevertheless, if pollination was maximised, the economic value of courgettes would increase by approximately £166/ha. This is similar to the apple variety 'Cox' which has an estimated pollination deficit of £146/ha in the UK (M. P. D. Garratt *et al.*, 2013). Interestingly, this was partly due to no significant difference between the yield of open pollinated and pollinator excluded flowers which demonstrate the ability of the Cox variety to set fruit in the absence of pollinators. However, the same study showed that the Gala variety had a much higher pollination deficit of £6,459/ha, due to an increased dependency of this variety on pollination and higher yield from hand-pollinated flowers. This demonstrates how important it is to include different pollinator dependency ratios based on inter-variety differences when performing economic valuations.

The price of courgettes used in this valuation (despite being a seasonal average) is likely to vary in response to the supply and demand of courgettes on the open market (Garratt *et al.*, 2013; Melathopoulos, Cutler and Tyedmers,

2015). Consequently, the economic value of insect pollination to courgettes presented in this study, tells us our actual and potential dependency on pollinators at this current time, rather than an absolute value. If pollinator populations were to decline in the UK, the supply of courgettes would decrease, which would increase demand (especially if alternative countries were also unable to meet demands). This would raise the price of courgettes on the open market and increase the total economic value of insect pollination.

Despite the relatively small pollination deficit in this study, spatial and temporal fluctuations in pollinator populations mean that it may still be beneficial for growers to improve pollination services, even if pollination deficits are due to natural variation in yield. A relatively quick and simple way of doing this is to use commercial bee species which are known to be effective pollinators of cucurbit crops (Artz and Nault, 2011; Petersen, Huseth and Nault, 2014) although see Figure A 5.1.

A longer-term but more sustainable option could be to enhance floral resources, a significant limiting factor in bee populations (Roulston and Goodell, 2011). Increased floral resources can attract pollinators to a site (Chapter 4) and provide resources for both managed and wild bees beyond that of the focal crop (Carvell *et al.*, 2007) (Chapter 5). Generally the effectiveness of these measures is moderated more by the surrounding landscape, rather than the size of the area planted (Heard *et al.*, 2007; Batáry *et al.*, 2011; Herbertsson *et al.*, 2018), with more simplistic landscapes showing greater yield increases than ones which already have good floral resources. As Cornwall already benefits from biodiverse hedgerows and generally smaller field sizes, availability of floral resources may be strongly influencing the high pollination rates observed in this

study. This would support the findings of Garratt *et al.*, (2017) which showed hedgerows to be a valuable source habitat for pollinators and natural enemies. Therefore, these findings provide a clear incentive for growers in this region to maintain and protect these habitats to ensure high and stable pollination services in the future. Growers may also benefit from using crop varieties which have been selectively bred to be fully parthenocarpic (currently not done by commercial growers of courgette), especially in combination with pollinator-supportive practices (Knapp *et al.*, 2016, Chapter 2).

Conclusion

Although confined to a single geographic region and variety, this study highlights the importance of pollination for improving yields, even when over half of the fruit set can still be achieved via parthenocarpy. Understanding a crop's demand for pollinators can help growers choose what varieties to use. In areas with lower visitation rates, potentially due to large fields or less natural habitat, growers may wish to increase the supply of pollinators. In doing so they may increase their agricultural resilience and further their economic advantage.

Realistic estimates of the amount of insect pollination required for optimum fruit set need to account for not only the variability in pollination deficit that might result from variable pollinator densities and environmental conditions, but also the variability in pollinator dependence between varieties of single crop species, for which there is currently little good evidence (Knapp *et al.*, 2016 (Chapter 2); Melathopoulos *et al.*, 2015, although see Garratt *et al.* 2014). In the wider context, discussion and strategies for improving horticultural crop production need to incorporate costs and benefits associated with different methods of

maximising pollination, whilst remembering that factors other than pollination also contribute to fruit set.

4. Pollinator visitation to mass-flowering courgette and co-flowering wild flowers: implications for pollination and pollinator conservation

In Chapter 3 I observed that whilst 56% of fruit was able to reach marketable size and shape without any pollination, pollination significantly increased yield at an estimated worth of £3,398 per ha. Since floral resources are a significant limiting factor in bee populations, growers may wish to boost the floral resources available to pollinators as a way of mitigating potential fluctuations in pollinator populations which may adversely affect their yield. Results are discussed in the context of management decisions which can be made to improve either crop pollination, or species conservation; or both.

Plate 7 Courgette co-flowering with wild flowers in Cornwall, UK. All study sites throughout this thesis were conventionally managed, however, the short picking interval of the crop means that little or no herbicide is used, resulting in a high abundance and diversity of wild flowers within and around the crop. This abundance of wild flowers so close to crop flowers inspired me to study how floral resources at different spatial scales, i.e. field scale: within the crop, around the crop, the whole field, and farm-scale: mass-flowering crops and semi-natural habitat may affect pollinator visitation to courgette.

Abstract

Managing the complex relationship between pollinators and their habitat requirements is of concern to growers of pollinator-dependent crop species, such as courgette (*Cucurbita pepo* L.). Naturally occurring wild flowers (i.e. agricultural weeds) offer a free, sustainable, and often underappreciated resource for pollinators, however they may compete with crop flowers for visits. To understand the extent to which floral resources mediate pollinator visitation to courgette flowers and courgette fields, data were collected at two spatial scales: field scale (in margins, and in the cropped area) and farm scale (500 m and 2000 m radii) for nine courgette fields across the UK. *Apis mellifera* L. (honeybees) and *Bombus* spp. (bumblebees) were the only pollinators observed to visit courgette flowers. Bumblebees were significantly more abundant on courgette flowers in fields with a greater species richness of wild flowers in the crop, whilst honeybees were significantly more abundant on courgette flowers in areas with less semi-natural habitat. For both honeybees and bumblebees, their abundance in field margins did not significantly reduce their abundance on courgette flowers, suggesting that wild flowers were not competing with courgette flowers for pollination services. Although solitary bees were not observed to visit courgette flowers, their abundance and species richness in courgette fields were significantly greater with more semi-natural habitat and a greater species richness of wild flowers. Therefore, allowing uncultivated areas around the crop to be colonised by species-rich wild flowers is an effective way of boosting the abundance of bumblebees - important visitors to courgette flowers, as well as the abundance and species richness of solitary bees - benefitting pollinator conservation.

Introduction

Agricultural expansion and intensification are thought to be responsible for reduced ecological functioning of farmland ecosystems. Sustainable management of these systems requires beneficial services such as pollination, pest control and nutrient cycling to be optimised whilst minimising any negative effects that they may have on other services. For example, effective biological weed control may reduce the availability of forage for pollinators and natural enemies, whilst areas planted for forage may compete with crop species for abiotic and biotic resources (Bretagnolle and Gaba, 2015). These factors are of particular concern to growers of pollinator-dependent crop species who must manage the complex relationship between pollinators, their habitat requirements and other ecosystem services important for determining yield (Bommarco, Kleijn and Potts, 2013).

Supplementing crop fields with managed bee species can bolster pollinator numbers (Garibaldi *et al.*, 2014) to prevent yield declines. This has been common practice for growers of *Cucurbitaceae* (cucurbits or gourds) (Free, 1993; Nerson, 2007), which have an 'essential' requirement for insect-mediated pollination (Klein *et al.* 2007, although see Knapp and Osborne 2017 (Chapter 3)). However, introducing managed pollinator species does not address one of the primary drivers of pollinator decline: loss of available forage (Vanbergen, 2013). Instead, improving the quantity and quality of pollen and nectar resources available for pollinators, and allowing areas to remain undisturbed for nesting, mating, and hibernation will benefit pollinator populations beyond that of the focal crop (Carvell *et al.*, 2007). At a field scale, floral resources can be increased by planting wild flower strips, allowing areas to be naturally colonised by wild flowers, and maintaining hedgerows. At a farm scale, proximity to, or

quantity of natural and semi-natural habitat can increase pollinator abundance as they spill into crop areas (Garibaldi *et al.*, 2011). For example, pollination services from native bees have been shown to be positively related to the proportion of natural and semi-natural habitat surrounding fields of watermelon (Kremen *et al.*, 2004) and pumpkin (Petersen, Jessica and Nault, 2014).

However, the effectiveness of field scale pollinator-supporting practices are often variable and greatly depend on the complexity of the habitat surrounding a crop field, with more simplistic landscapes generally showing greater increases in pollinator species richness after interventions than in complex landscapes (Batáry, Báldi, Kleijn, & Tscharntke, 2011; Scheper *et al.*, 2013). At a field scale, providing additional floral resources within fields (such as wild flowers) may attract or distract pollinators from the focal crop as they facilitate or compete for pollination services (Nicholls and Altieri, 2013). At a farm scale, the area of mass-flowering crops may 'dilute' pollinator densities if large, or 'concentrate' pollinator densities if small (Holzschuh *et al.* 2016). This will be especially pronounced if additional food and nesting sites are not provided, meaning that pollinators move transiently between available forage rather than increasing their population size (Holzschuh *et al.* 2016, see also Chapter 5). The complexity of field and farm-scale resources becomes further complicated when species-level responses are taken into consideration. Increasing the proximity of forage to suitable nesting sites may be more important for 'door step foragers' such as *Bombus muscorum* L., *B. pascuorum* Sc. and *B. lapidarius* L. which are known to forage close to their nests (Osborne *et al.*, 2008). Other species with longer flight distances (such as *B. terrestris* L. and *Apis mellifera* L.) are found in high abundance in large, intensively farmed fields, far away from available nest sites (Osborne *et al.*, 2008). Since previous research has

shown that *B. terrestris* and *A. mellifera* can fulfil the pollination requirements of courgette (in UK: Knapp and Osborne 2017) and *B. impatiens* and *A. mellifera* of pumpkin (in USA: Petersen *et al.* 2013), increasing the proximity of forage to suitable nesting sites may be less important to cucurbit crops because they are primarily serviced by long range, generalist pollinators. This highlights the need to match pollinator-supportive management practices with crops' individual requirements for pollination, since an increase in pollinator species richness may not necessarily be required for yield to be improved (Kleijn *et al.*, 2015; Winfree *et al.*, 2015).

The positive, mediating effect of forage availability on pollinator visitation and crop yield has been shown in pumpkin (Petersen and Nault 2014), strawberry (Connelly *et al.* 2015), poppy (Hardman *et al.* 2016), and coffee (Saturni *et al.* 2016). Likewise, several studies have looked at forage availability at different spatial scales, in terms of patch size (Heard *et al.*, 2007), and field versus farm scale allocation of resources (Hardman *et al.*, 2016). However, these analyses do not differentiate between pollinator visitors to the focal crop and all other pollinator species, thus increases in pollinator numbers may not necessarily benefit crop pollination.

This study focuses on pollinator visitation to mass-flowering courgette (*Cucurbita pepo* L.) and co-flowering wild flowers to further understand the extent to which available forage (at different spatial scales) may improve pollination and/or pollinator conservation in crop fields. To do this we ask: (1) Which pollinator species visit courgette flowers and which ones visit co-flowering wild flowers in crop fields? (2) How does forage availability and pollinator presence in field margins affect pollinator visitation to courgette

flowers? And (3) How does forage availability affect the abundance and species richness of pollinators which do not visit courgette?

Materials and methods

Study sites

In 2016, nine courgette (var. Tosca) fields were surveyed across the United Kingdom in Cornwall (five sites), Worcestershire (two sites) and Cambridgeshire (two sites), to represent a range of climatic variables (Figure 4.1). Tosca is a popular courgette variety in the United Kingdom, representing 37.9% of the market share (P.E. Simmons and Son, personal communication 1 April 2017).

All courgettes were grown conventionally in outdoor conditions. Little or no herbicide is used on courgette fields due to the short picking intervals of the crop (P.E. Simmons and Son, personal communication 1st November 2017), which results in a high abundance and species richness of wild flowers within the crop. The fields were managed by five horticultural growing companies; with similar farming practices checked using questionnaires. Sites differed slightly in the number of herbicide (0-5 applications, median = 2.5), fungicide (0-4 applications, median = 2) and fertiliser applications (1-5 applications, median = 2.5).

Fields (average field size of 8.5 ± 1.9 ha (*SE*)) were situated at least 2 km apart so that pollinator communities are unlikely to be shared between fields (Vaissière, 2010), and each field was visited three times between 1st June and 31st July, during courgette blooming. Eight 50 m transects per field were established; four along each field margin and four within the crop (from the edge of the crop to the centre, 25 m apart) (Figure 4.1).

Pollinator surveys

Transects were walked at a steady pace (~5 minutes each) with observations made 1 m either side and in front of the recorder (over a length of 50 m). All bee species and the plant species they were on (if feeding or pollen collecting) were recorded to species level. Sampling was conducted between 08:00 and 10:00 h (when flowers were open) on sunny to partly cloudy days.

Halfway along each transect, one pan trap (15 cm diameter plastic bowl sprayed with yellow UV paint) was placed 20 cm off the ground (average height of courgette flowers) for 24 hours (Figure 4.1). Traps were filled with dilute scentless soapy water. All bees and hoverflies were identified to species level in the laboratory. Pollinator transects, and pan traps were analysed separately (transects were used to calculate the number pollinators on courgette flowers and pan traps were used to estimate the abundance and species richness of solitary bees (non-courgette pollinators)). Using this combination of survey techniques gave a better representation of overall species richness and an insight into plant-pollinator interactions (Westphal *et al.*, 2008). In addition, the importance of different floral resources for pollinators was assessed by constructing network diagrams of pollinator/ flower interactions in courgette fields, divided into field margins and the cropped area itself.

Floral surveys

Wild flower surveys were conducted at the same time and location as the pollinator transects (Figure 4.1). To calculate the availability of non-crop floral resources (wild flowers), the numbers of open flowers or floral units (in the case of composites) of insect rewarding plant species (defined in Hardman *et al.* (2016)) were recorded.

Landscape variables

Since semi-natural habitat and mass-flowering crops can increase pollinator populations (Westphal, Steffan-Dewenter and Tscharntke, 2003; Kremen *et al.*, 2004), the total area of semi-natural habitat (woodlands and heathland) and mass-flowering crops (courgette, oilseed rape, and field beans) were calculated in 500 m and 2000 m radii of each field (m²) using CEH Land Cover® plus: Crops (for information on annual crop types) and Land Cover 2007 (for information on habitat types (Centre for Ecology and Hydrology, 2011, 2016) using ArcGIS 10.2.2.

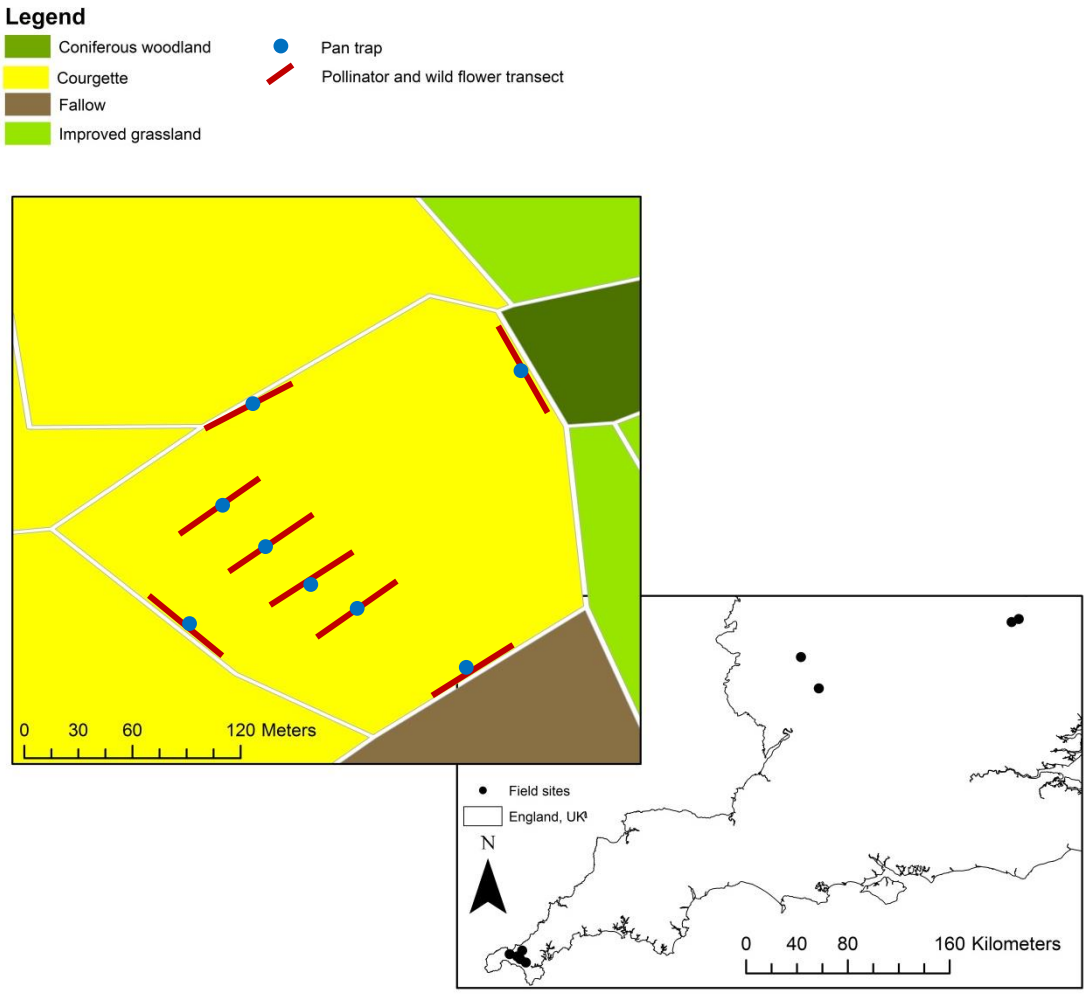

Figure 4.1 One field site showing the locations of pollinator and wild flower transects and pan traps. The location of all field sites (five in Cornwall, two in Worcestershire and two in Cambridgeshire) in 2016 are also shown.

Statistical analysis

All analyses were carried out using R (R Core Team, 2017). Linear mixed effect models (Bates *et al.*, 2015) were used to investigate: 1) the abundance of bee species observed visiting courgette flowers (honeybees and bumblebees) and 2) the abundance and species richness of other bee species (solitary bees) which were not observed visiting courgette flowers.

For each response variable, a set of candidate models were constructed using rescaled (between 0 and 1) predictor variables. For courgette visitors (honeybees or bumblebees), the full fixed effects model contained the abundance of the same species on field margins (either honeybees or bumblebees depending on the response variable), abundance of wild flowers per crop transect, species richness of flowers per crop transect, total number of wild flowers per field (sum of wild flowers in the margins and cropped area), area of semi-natural habitat (m^2 in 500 m and 2000 m radii), area of mass-flowering crops (m^2 in 500 m and 2000 m radii), field size and the interaction between mass-flowering crops or semi-natural habitat and wild flowers per crop transect and wild flowers per field. Region and field size were entered as random effects, and a null model including random effects only included in the candidate model set. Abundance and species richness of floral resources were always tested in separate models due to collinearity, as were semi-natural habitat and mass-flowering crops (at 500 m and 2000 m radii) (Figure A 3.2).

Non-courgette visitors (solitary bees) were analysed in similar models but included data from both margins and cropped area (per transect) in the response variable. Transect location (margin or cropped area) was added as a fixed effect.

For each stage of the analysis, all possible combinations of the full model (all fixed effects) were compared to the null intercept-only model using Akaike's Information Criterion for small sample sizes (AICc) (Barton, 2017), with the lowest AICc score defining the model that best describes the data (Symonds and Moussalli, 2011).

Models with a ΔAICc ≤2 were examined (model averaging was not carried out due to correlated predictor variables) and the most parsimonious of these (the one with the lowest AICc) further checked for multicollinearity using variance inflation factors (VIFs) (Fox & Weisberg, 2011). All covariates had VIFs <5. The full and best fitting models were visually checked to meet model assumptions (homogeneity of variance, normal distribution of residuals and the presence of influential values). The significance of fixed effects in the best fitting model were tested using summary *T* and *P*-values (Kuznetsova, Brockhoff and Christensen, 2016).

Results

Pollinator community

Overall, a total of 958 pollinators of 53 different species were recorded during this study. All 53 species were recorded in pan traps and 9 species were recorded on pollinator transects (Appendix 3.3). Pollinator abundance was comprised of 57% hoverflies, 31% solitary bees and 12% social bees (honeybees or bumblebees) in pan traps, and 84% social bees and 16% hoverflies on pollinator transects. Nonetheless, three bee species and one hoverfly species accounted for 77% of all pollinator records: *Apis mellifera* (n = 379), *Bombus terrestris/ lucorum* (n=196), *Bombus lapidarius* (n = 55), and *Eupodes corollae* F. (n = 110).

An average of 46% of pollinator species occurred in both the margin and within the crop, which included 6 bumblebee, 8 solitary bee, and 9 hoverfly species. Honeybees (and to a lesser extent bumblebees) were more abundant in the cropped area, whilst solitary bees were more abundant on field margins (Figure A 3.3a). Courgette was the most visited floral resource with 52% of all flower visits recorded (including margins) being to staminate or pistillate courgette flowers. The next most frequently visited flower species were *Sinapsis arvensis* and *Cirsium palustre* which received just 8% and 6% of pollinator visits respectively. Out of all pollinator species visiting courgette flowers, *A. mellifera* and *B. terrestris* were the most abundant (Figure 4.2~~Figure 4.2~~, see also Figure 5.4 Chapter 5). However, whilst *B. terrestris* visited both staminate and pistillate flowers (staminate flowers = 45 visits, pistillate flowers = 47 visits), *A. mellifera* showed a preference for pistillate flowers (staminate flowers = 270 visits, pistillate flowers = 13 visits) (Figure 4.2~~Figure 4.2~~, see also Figure 5.4 Chapter 5).

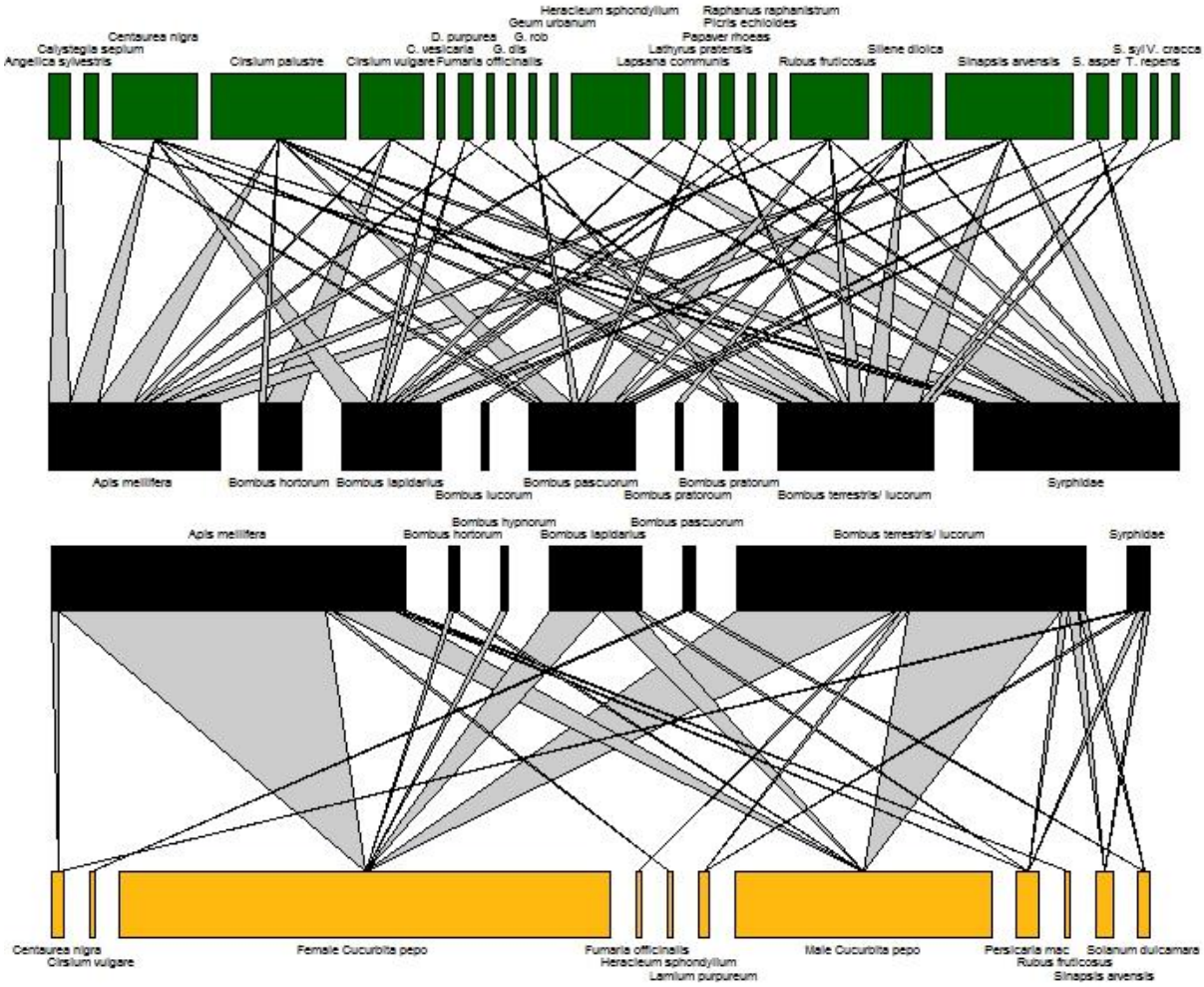

Figure 4.2 Pollinator communities of courgette fields divided into field margins (green) and the cropped area (yellow). Pollinator communities for field margins are upside-down for easy comparison with pollinator communities in the cropped area. The width of bars indicates the proportion of species which were recorded during pollinator transects feeding on a particular plant species. Full plant species names for labels are: *Angelica sylvestris, Crepis vesicaria, Digitalis purpurea, Geranium dissectum, Geranium robertianum, Sonchus asper, Stachys sylvatica, Trifolium repens, Viccia cracca.*

Floral resources

Across all regions floral species richness was greater in field margins than in the crop (mean margin = 29 ± 4 *SE*, crop = 12 ± 2 *SE*) and floral abundance was generally higher in field margins than in the crop (mean margin = 23,273 ± 5,365 *SE*, crop = 10,529 ± 4,838 *SE*) flowers (summed per transect) (Figure A 3.3b-c). The amount of semi-natural habitat varied from 0 - 13% (median = 3%)

in a 500 m buffer and 1 - 17% (median = 9%) in a 2000 m buffer. The amount of mass-flowering crop~~s~~ varied from 14 - 37% (median = 23%) in a 500 m radius and 2 - 13% (median = 3%) in a 2000 m radius. 94% of mass-flowering crop in the landscape was courgette and was therefore flowering simultaneously with courgette at the study sites.

Impact of floral resources on courgette pollinators

Honeybee abundance on courgette flowers was best predicted by a decrease in semi-natural habitat in a 2000 m radius (m²) (β = -0.10 ± 0.13, *T* = -7.44, *P* = 0.023).

Impact of floral resources on solitary bees

The best fitting model for solitary bee abundance included transect location (margin or crop), an increase in field size, species richness of wild flowers in fields, semi-natural habitat in a 2000 m radius and the interaction between wild flower species richness and semi-natural habitat (Table 4.2~~Table 4.2~~). Solitary bee abundance was positively correlated to wild flower species richness (summed per transect) (β = 0.93 ± 0.33, *T* = 2.78, *P* = 0. 007) and there was a significant interaction between floral species richness (summed per transect) and semi-natural habitat in a 2000m radius (m²) (β = -1.46 ± 0.35, *T* = -4.24, *P* < 0.001), with an increase in wildflower species richness positively correlated to solitary bee numbers in landscapes with low semi-natural habitat (Figure 4.4). For all other predictors in the best fitting model the relationship was not significant (margin transect location (β = 0.76 ± 0.5, *T* = 1.50, *P* = 0.14); field size (Ha) -β = 0.72 ± 0.4, *T* = 1.63, *P* = 0.11); semi-natural habitat in a 2000 m radius (m²) β = -0.23 ± 0.37, *T* = -0.61, *P* = 0.55.

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The best fitting model for solitary bee species richness included location (margin or crop), species richness of wild flowers in fields, semi-natural habitat in a 2000 m radius and the interaction between wild flower species richness and semi-natural habitat (Table 4.2~~Table 4.2~~). Solitary bee species richness was significantly greater on field margins (β = 0.74 ± 0.32, *T* = 2.35, *P* = 0.02) (Figure A 3.3a) and wildflower species richness had a greater impact in areas with less semi-natural habitat (overall interaction effect size β = -0.57 ± 0.2, *T* = -2.35, *P* = 0.02, Figure 4.4b). There was no significant relationship with wild flower species richness (summed per transect) (β = -0.33 ± 1.9, *T* = 1.88, *P* = 0.07), or semi-natural habitat in a 2000 m radius (m²) (β = -0.33 ± 0.19, *T* = -1.69, *P* = 0.12).

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Table 4.1 Top three best fitting models (AICc Δ< 2) describing the impact of field size and floral resources (at a local, and landscape scale) for 1a) honeybees, and 2a) bumblebees which were observed visiting courgette flowers. Only coefficient estimates for continuous fixed effects which occur in the best fitting models are presented in the table. Global models: Field size + (Abundance of wild flowers in the cropped area + Species richness of wild flowers in the cropped area) +Total number of wild flowers in the field + Edge honeybee abundance or Edge bumblebee abundance + (Semi-natural Habitat (SNH) 500m + Semi-natural Habitat 2000m) + (Mass-flowering Crops (MFC) 500m + Mass-flowering Crops 2000m) + *all measures of wild flowers* * MFC/SNH. Brackets indicate where due to multicollinearity only one predictor variable for local floral resources and one variable for semi-natural habitat and mass-flowering crops were entered into the model. The R^2 (a measure of overall model fit) is divided into marginal R^2 (R^2_m) and conditional R^2 (R^2_C) following Nakagawa and Schielzeth (2013).

	Intercept	Edge bumblebee abundance	Edge honeybee abundance	Species richness of wild flowers in the cropped area/ transect	SNH 2000 m	df	AICc	ΔAICc	Weight	R^2_m	R^2_c
1a) Honeybee abundance on courgette flowers (per transect)	1.61	-		-0.22	-1.01	6	86.60	0.00	0.13	0.73	0.73
	1.72	-	-0.06		-1.04	6	87.23	0.63	0.10	0.15	0.65
	1.61	-			-1.06	5	87.27	0.68	0.09	0.70	0.71
1b) Bumblebee abundance on courgette flowers (per transect)	1.53		-	3.06		5	127.50	0.00	0.15	0.09	0.80
	1.79	-0.06	-	2.91		5	128.84	1.35	0.08	0.09	0.80

Table 4.2 Top three best fitting models (AICc Δ< 2) describing the impact of field size and floral resources (at a local, and landscape scale) for 2a) solitary bee abundance, and 2b) solitary bee species richness. Only coefficient estimates for continuous fixed effects which occur in the best fitting models are presented in the table. Global models contained: Transect location + Field size +Total number of wild flowers in the field + (Semi-natural Habitat (SNH) 500m + Semi-natural Habitat 2000m) + (Mass-flowering Crops (MFC) 500m + Mass-flowering Crops 2000m) + Total number of wild flowers in the field * MFC/SNH. Brackets indicate where due to multicollinearity only one predictor variable for local floral resources and one variable for semi-natural habitat and mass-flowering crops were entered into the model. The R^2 (a measure of overall model fit) is divided into marginal R^2 (R^2 m) and conditional R^2 (R^2C) following Nakagawa and Schielzeth (2013).

	Intercept	Location	Field size	Abundance of wild flowers	Species richness of wild flowers	SNH 2000 m * Species richness of wild flowers/ transect	df	AICc	ΔAICc	Weight	R^2_m	R^2_c
2a) Solitary bee abundance summed from pan traps (per transect)	-3.53	+ Margin	2.36		13.43	-20.96	9	294.79	0.00	0.39	0.31	0.31
	-1.49	+ Margin			11.42	-19.80	8	295.19	0.40	0.32	0.28	0.28
2b) Solitary bee species richness summed from pan traps (per transect)	-0.45	+ Margin			5.31	-8.28	8	234.17	0.00	0.07	0.21	0.21
	0.50	+ Margin					5	234.53	0.35	0.06	0.11	0.16
	0.36	+ Margin		0.97			6	234.65	0.48	0.05	0.14	0.20

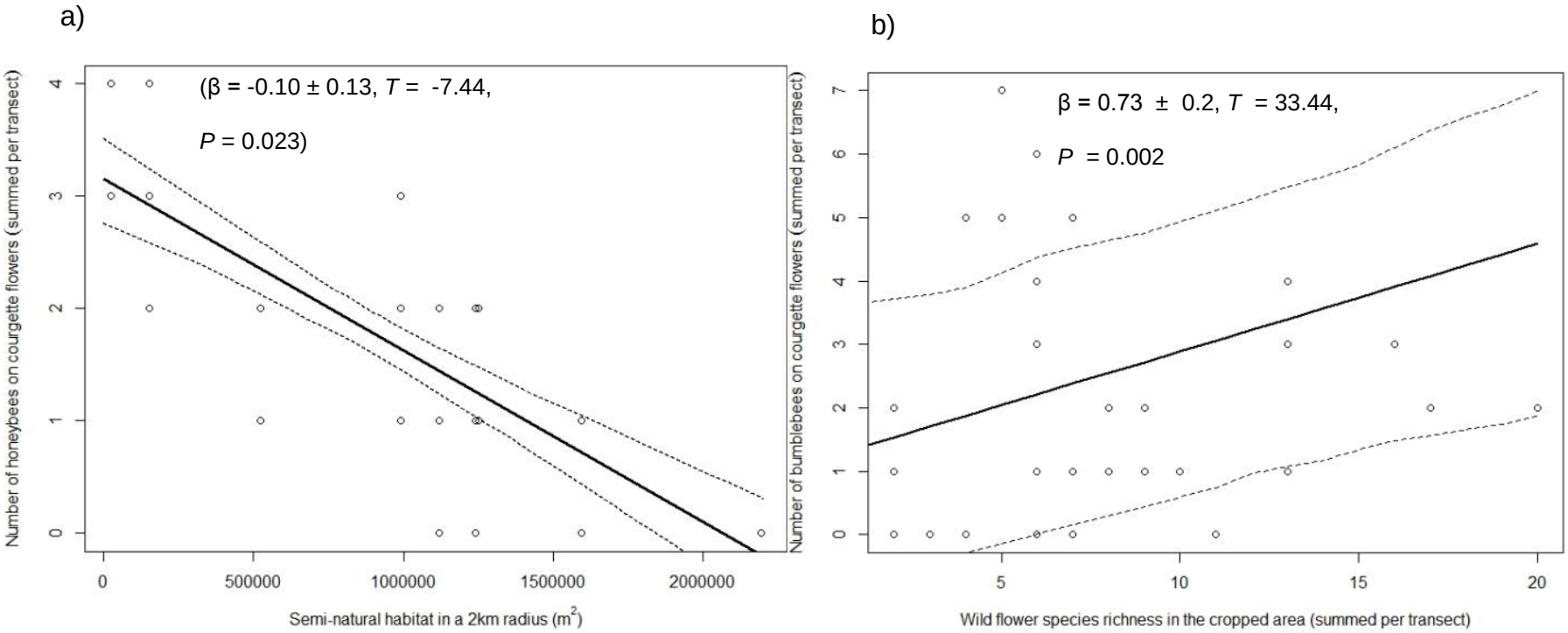

Figure 4.3 Significant effects from best fitting models (lowest AICc) of a) honeybee abundance on courgette flowers (summed per transect) in relation to semi-natural habitat in a 2000 m radius (m²), and b) bumblebee abundance on courgette flowers (summed per transect) in relation to the species richness of wild flowers in the cropped area. Solid lines are predicted values from linear models and dashed lines 95% confidence intervals calculated via boot strapping of 200 simulations.

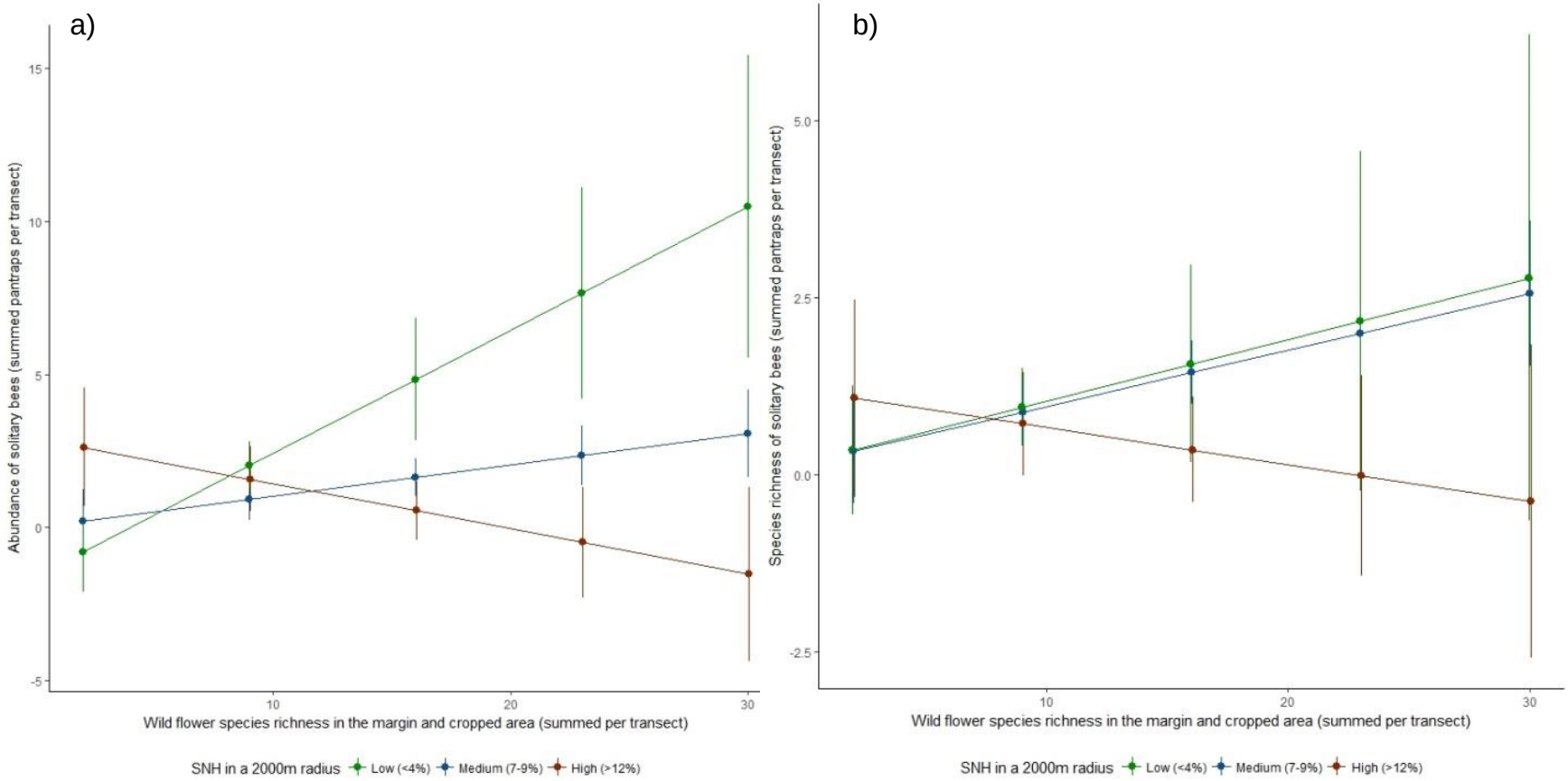

Figure 4.4 Significant effects from best fitting models (lowest AICc) of a) Abundance of solitary bees (summed from pan traps (per transect location)), and b) Species richness of solitary bees (summed from pan traps (per transect)) in relation to the species richness of wild flowers in the margin and cropped area (summed per transect) and semi-natural habitat in a 2000 m radius (m^2).

Discussion

Allocation of floral resources to increase pollinator abundance is the primary basis for pollinator-supportive land management; however, less is known about how the scale of floral resources (field or farm) affects different pollinators of a focal crop. By understanding the dynamics more fully, pollinator-supportive land management can be targeted *either* to pollinator species which are known to visit the focal crop, with the aim of improving crop pollination, *or* to other pollinator species more generally, with the aim of improving species conservation; or both.

Community networks of pollinators in courgette fields show that whilst field margins support a greater number of pollinator species, many of these species also occur within the cropped area, suggesting movement between the two areas. Of these species, *A. mellifera* and *B. terrestris* were the most abundant and occur in their greatest numbers within the cropped area. These two species also show a preference for courgette flowers, with *B. terrestris* showing a more equal preference than *A. mellifera* for male and female courgette flowers which may affect their relative effectiveness as pollinators (Ne'eman *et al.*, 2010; Artz, Hsu and Nault, 2011). These networks also show that several widespread, resilient species (rather than species-rich communities) are the main pollinator visitors to courgette (see also Chapter 2). Although these species respond positively to increased provision of floral resources their populations can be increased by beekeepers/ farmers introducing managed colonies to an area. Honeybee density on crop flowers may also be intensified by their *en masse* recruitment to areas of good forage (von Frisch, 1967) and beekeepers may have placed more honeybee colonies into areas with more mass-flowering crop. Bumblebee abundance on courgette flowers was correlated to an increase in

species richness of wild flowers in the cropped area. As bumblebees have been observed to stay constant to an area of good forage (Osborne et al., 1999) it is likely that they are foraging between wild and crop flowers (due to their phenology) at a specific locality (see also Figure 5.3b, Chapter 5).

Importantly, the abundance of honeybees or bumblebees on field margins did not influence their abundance on courgette flowers. This suggests that wild flowers are not competing with crop flowers for pollinator visitation, supporting findings from almond orchards (Lundin *et al.*, 2017). Nonetheless, honeybee foragers were more abundant on courgette flowers in areas with less semi-natural habitat; and where there was lower species richness of wild flowers in the cropped area, suggesting that courgette may compete with co-flowering wild flowers for honeybee visits, although this was not statistically significant. Whilst In the short-term wild flowers within the crop may compete with courgette for pollination services, these wild flowers may improve pollinator populations and thus crop pollination in the long-term (Mitchell *et al.*, 2009). Unfortunately, no information is available on the abundance of honeybee hives or the location of apiaries within a 10km resolution of study sites. Therefore, it is impossible to determine if honeybee forager abundance is moderated by the amount of semi-natural habitat in the landscape or beekeeping practices. The overall abundance of honeybee foragers at a site did not influence bumblebee abundance at courgette flowers and vice versa, nor interact with other landscape features, suggesting that these species are not in competition with each other for common resources and that the surrounding landscape is not moderating any potential competition between species (Herbertsson *et al.*, 2016).

Floral species richness was more important for predicting solitary bee abundance and species richness (non-courgette visitors) in sites with less semi-natural habitat, suggesting that a higher species richness of wild flowers is needed if courgette fields are in areas with less semi-natural habitat. Kennedy *et al.*, (2013), also showed that in conventional fields, bee abundance and richness benefit from increased landscape complexity suggesting that pollinator conservation on farmland will ultimately depend on the presence of high quality semi-natural habitat (Kennedy *et al.*, 2013; Beduschi *et al.*, 2018). Wild flowers within fields have been shown to be more effective at increasing pollinator abundance and species richness in simple landscapes (with less semi-natural habitat) compared to complex landscapes (Scheper, Holzschuh, Kuussaari, Potts, Rundlöf, Smith, Kleijn, *et al.*, 2013; Herbertsson *et al.*, 2018). Since Scheper et al. (2013) and this study only analysed foraging individuals it is impossible to determine if differences in pollinator abundance and species richness between study sites were due to the transient movement of species between areas of forage, or due to an actual population increase.

The abundance and species richness of solitary bees were also higher in the margin (where there is a higher abundance and species richness of wild flowers) than the cropped area. These findings are likely due to solitary bees having broader dietary and nesting requirements, and much smaller foraging ranges (150 - 600 m) than honeybees and *B. terrestris* (the predominant bumblebee species) (Gathmann and Tscharntke, 2002; Greenleaf *et al.*, 2007). Floral resources on the field margin also increased the abundance of *Eupodes corollae*, a hoverfly species whose larval stage is a natural predator of soft-bodied arthropods such as aphids (Gomez-Polo et al. 2014). Therefore, provisioning floral resources at a field scale may increase pollinator abundance,

species richness (Appendix 3.3), yield (Appendix 3.1), and natural enemies of pests (Blaauw and Isaacs, 2014; Wood *et al.*, 2015; Garratt *et al.*, 2017).

Conclusion

This study shows that it is important to know which pollinator species visit crop or wild flowers so that management decisions can be made to improve either crop pollination, *or* species conservation; or both (Kleijn *et al.*, 2015). These findings demonstrate that allowing uncultivated areas around the crop to be colonised by species-rich wild flowers is an effective way of boosting the abundance of bumblebees - important pollinators of courgette (Chapter 3 and Chapter 5), as well as the abundance and species richness of solitary bees – important pollinators of other crop and wild flower species which may be co-flowering in the landscape. Thus, provisioning floral resources may benefit pollination services and pollinator conservation.

5. *Bombus terrestris* in a mass-flowering pollinator-dependent crop: A mutualistic relationship?

Pollinator dependence and pollination deficit (Chapter 3) and factors that may improve pollinator visitation (Chapter 4) are primarily concerned with what pollinators can do for courgette. However, courgette flowers may also help pollinators by providing abundant sources of nectar and pollen in the landscape which can boost population growth. *B. terrestris* was the focal pollinator species for this study due to its natural abundance in courgette fields (Chapter 3), preference for courgette flowers (Chapter 4), and contribution toward courgette yield (Chapter 3 and Appendix 3.1).

Plate 8 Sampling pollen loads from returning foragers caught in 'forager trap modules' (Osborne, Martin, Carreck, *et al.*, 2008) which were placed onto commercial colonies of *B. terrestris* positioned in courgette fields. Two years of surveying prior to this study had frequently shown *B. terrestris* collecting courgette nectar, but never courgette pollen. Photograph by Daphne Wong.

Abstract

Bumblebees (*Bombus* spp.) rely on an abundant and diverse selection of floral resources to meet their nutritional requirements. However, mass-flowering crops are frequently overlooked as a nutritional resource for bumblebees, despite growers expecting bees to primarily forage on the crops to deliver pollination services. This study explores the mutualistic relationship between *Bombus terrestris* L. (buff-tailed bumblebee), a common species in European farmland, and the mass-flowering crop courgette (*Cucurbita pepo* L.) to see how effective *B. terrestris* is at pollinating courgette and in return how courgette may affect *B. terrestris* colony dynamics. By combining empirical data on nectar and pollen availability with model simulations (using the novel bumblebee model *Bumble*-BEEHAVE) we were able to quantify and simulate for the first time, the importance of courgette as a mass-flowering forage resource for bumblebees. Courgette provides vast quantities of nectar and pollen which ensures a high visitation rate and pollination potential of *B. terrestris*. Whilst *B. terrestris* showed a strong fidelity to courgette flowers for nectar, courgette pollen was not found in any pollen loads from returning foragers. Nonetheless, model simulations showed that early season courgette increased the number of hibernating queens, colonies, and adult workers (in the modelled landscape), but not colony size or nectar or pollen stores per bee. Courgette has the potential to improve bumblebee population dynamics however, bees can only benefit from this transient nectar source if alternative floral resources (particularly pollen) are also available to fulfil bees' nutritional requirements in space and time. Therefore, providing additional forage resources could simultaneously improve pollination services *and* bumblebee populations.

Introduction

Loss of floral resources due to changes in land management is generally thought to be the primary driver of reported declines in pollinator populations (Brown and Paxton, 2009; Potts *et al.*, 2010; Baude *et al.*, 2016). This is because generalist flower visitors such as bumblebees (*Bombus* spp.) rely on an abundant and diverse selection of floral resources for nectar and pollen to meet their energy requirements: nectar is rich in sugars, a source fuel, and pollen is rich in protein which is essential for growth and development (Rotheray, Osborne and Goulson, 2017).

Mass-flowering crops are frequently overlooked as a nutritional resource for bumblebees, despite growers expecting bees to primarily forage on the crops to deliver pollination services (Pufal, Steffan-Dewenter & Klein 2017). This is the case for growers of courgette (*Cucurbita pepo* L.) where pollination, particularly by bumblebee species has been shown to increase yield by 39% (Knapp & Osborne 2017, Chapter 3). Indeed *Bombus impatiens* C. (a North American species) has been observed to be a highly effective pollinator in *Cucurbita* crops, depositing more than three times the number of pollen grains per stigma compared to *A. mellifera* L. and *Peponapis pruinosa* S. (Artz and Nault, 2011). Quantifying the effectiveness of individual pollinator species can help growers target their pollination management to species most likely to increase yields (Ne'eman *et al.*, 2010).

Whilst mass-flowering crops may enhance pollinator densities (Westphal, Steffan-Dewenter and Tscharntke, 2003) it is largely unknown if this is due to a transient movement of bees between patches of forage or due to an actual increase in colony growth (Holzschuh *et al.*, 2016). This is because mass-

flowering crops only provide temporary pulses of nectar and pollen unlike natural areas (with higher floral species richness) which are able to provide resources that are more stable over time (Montero-Castaño, Ortiz-Sánchez and Vilà, 2016). Nonetheless, intense flowering periods and large area of mass-flowering crops in the landscape may still benefit pollinators spatially and temporally, potentially boosting bee populations (and not just forager numbers) *and* pollination.

Since accurately studying bumblebee colony development in a field setting can be difficult (Westphal, Steffan-Dewenter and Tscharntke, 2009; Wood *et al.*, 2015), this study uses an *in-silico* approach to simulate the population dynamics of *Bombus terrestris* L. in landscapes with and without courgette fields using the agent-based model *Bumble*-BEEHAVE (Becher & Twiston-Davies *et al.* 2018). Although other Bumblebee models exist (Olsson *et al.*, 2015; Crone and Williams, 2016; Häussler *et al.*, 2017), *Bumble*-BEEHAVE is uniquely able to simulate the effects of multifactorial stressors on bumblebee survival at individual, colony and population levels on a daily basis, based on nectar and pollen sources which are approximated from real landscape maps of study sites.

Simulations were run in BEE-STEWARD (www.beehave-model.net), a software tool that combines in a user-friendly way the bumblebee model *Bumble*-BEEHAVE and the landscape defining features of BEESCOUT (Becher *et al.*, 2016). BEESCOUT was developed as the landscape module for the honeybee model BEEHAVE (Becher *et al.*, 2014) and for *Bumble-BEEHAVE* (Becher and Twiston-Davies *et al.* 2018), and creates input files from images of landscape maps. These input files define the number and specification of food sources

such as, nectar and pollen, flowering phenology etc. and therefore represent landscapes in the BEEHAVE and *Bumble*-BEEHAVE models. BEE-STEWARDS' interface also enables users to simulate the effects that different management options, such as changing crop types will have on bumblebee population dynamics.

This study explores the mutualistic relationship between *B. terrestris*, a common bumblebee visitor to courgette fields in the United Kingdom (Knapp & Osborne 2017, Chapters 3 and 4), and the mass-flowering crop courgette to ask: (1) How much pollen and nectar do courgette crops provide? (2) Is *B. terrestris* an effective pollinator of courgette? And (3) how does courgette affect *B. terrestris* colony development at a landscape scale (using *Bumble*-BEEHAVE)?

To answer these questions we quantified the amount of courgette nectar and pollen available every 90 minutes, since these floral rewards directly influence the potential pollination efficiency of *B. terrestris* (measured as bee abundance at courgette flowers (and wild flowers) and the number of pollen grains transferred on their bodies), and therefore, the pollination of courgette (measured as the number of pollen grains deposited onto stigmas and yield (from all flower visitors)) (Figure 5.1).

In addition, the amount of nectar and pollen produced by courgette over 24 hours and the proportion of courgette pollen in *B. terrestris'* diet (from pollen loads sampled from returning commercial *B. terrestris* foragers) was quantified. Since the amount of food and nesting resources (e.g. area of courgette and semi-natural habitat) in the landscape may affect bumblebee colony development, each study site was mapped. Field data on landscapes, and

nectar and pollen quantities were used to parameterise the simulations (Figure 5.1).

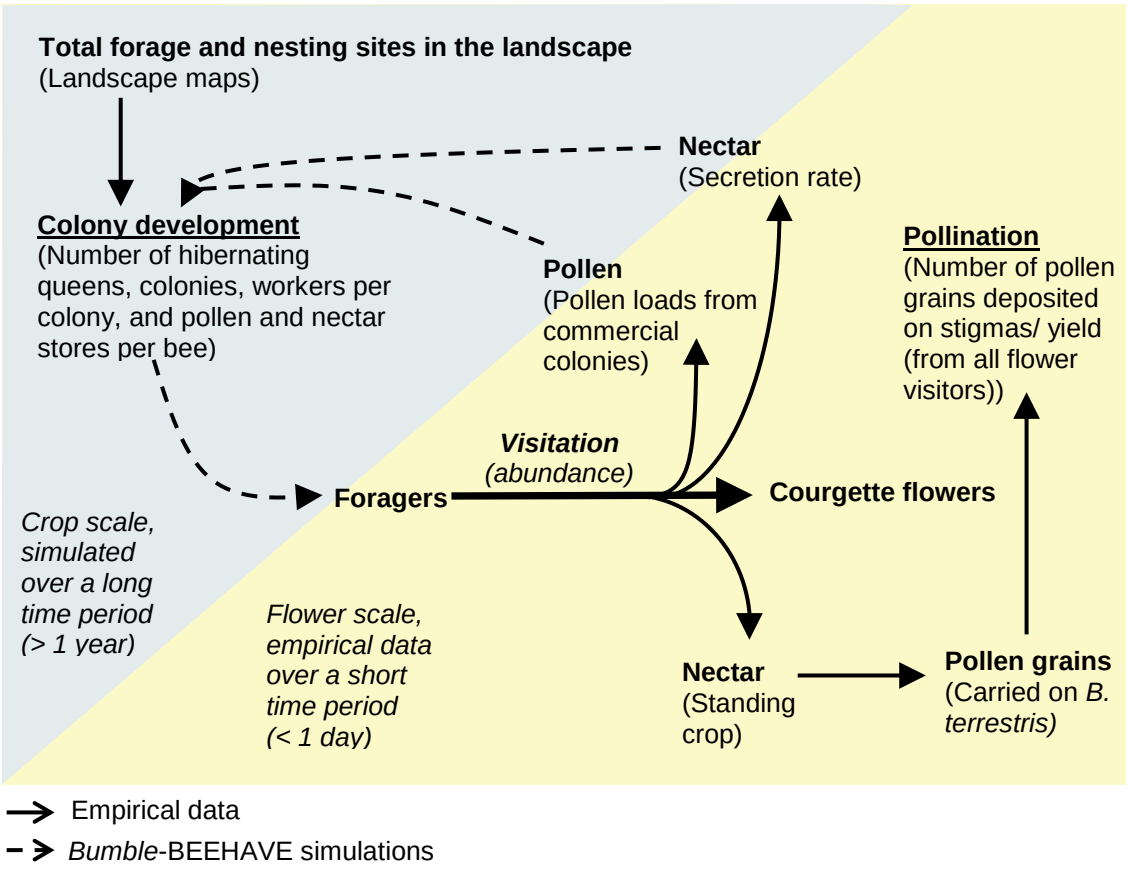

Figure 5.1 Concept explored in this study of the mutualistic relationship between *B. terrestris* and courgette. Solid arrows show where empirical data were collected, and dashed arrows show where results were created from *Bumble*-BEEHAVE simulations (using BEE-STEWARD software). Methods for each stage are in parenthesis.

Materials and methods

Study species

Courgette is monoecious with predominate staminate flowers until pistillate flowers gradually dominate over a season. Within a single day, both types of flower start opening around 05:30 before closing around 12:00 h on the same

day, and they do not open again. Although flower anthesis and senescence varies slightly depending on the climate, it is not thought to be directly affected by climatic events such as rainfall (Nepi, Massimo and Pacini, 1993).

In the United Kingdom, courgette is usually grown over two cropping periods (flowering and harvesting lasting around 5 weeks) at two separate sites (often several kilometres apart) to ensure a constant supply of courgette from the beginning of June until the end of August. Hereafter the first cropping period is referred to as 'early courgette' and the second cropping period is referred to as 'late courgette'.

Although all bee species visiting courgette were recorded during pollinator surveys, *Bombus terrestris* was the focus of this study because of their natural abundance at study sites and availability as commercial colonies (Biobest Biological Systems, Belgium) which were required to quantify the proportion of courgette pollen in *B. terrestris'* diet (Figure 5.1). Colonies were placed in each field (with sugar water but no additional pollen) at a density of three colonies per field.

Study sites

The empirical data for this study (Figure 5.1) were collected in 19 courgette (var. 'Tosca') fields in Cornwall, UK from the beginning of June until the end of August in 2016 (5 fields) and 2017 (14 fields). Each field (average field size of 3.6 ± 0.3 ha *SE*) was situated at least 2 km from any other courgette field so that pollinator communities were unlikely to be shared between fields (Vaissière, 2010).

All courgettes were grown conventionally in outdoor (as opposed to protected) conditions in fields surrounded by species-rich hedgerows, where little or no

herbicide was used due to the short picking intervals of the crop (P.E. Simmons and Son, personal communication 1st November 2017). This meant that there was a high abundance and species richness of wild flowers within and around the crop.

Quantifying nectar and pollen resources in courgette flowers (2017)

The standing crop of nectar, i.e. the amount of nectar available to visiting insects at a given time, was quantified to show how the volume of courgette nectar changed within a day. On the other hand, the 24 hour secretion rate, i.e. the overall amount of nectar produced by a flower over 24 hours was quantified to show the maximum nectar resource available from all courgette flowers within a landscape per day, which was needed to parameterise *Bumble*-BEEHAVE (Figure 5.1).

For each flower, full access to the nectary was achieved by removing the stigma or stamen with a scalpel. The standing crop of nectar was calculated from 50 staminate and 50 pistillate flowers every 90 minutes from 05:30 to 12:00 h over five days (10 staminate and 10 pistillate flowers per time point per day). This volume is likely less than when the same flower is repeatedly surveyed due to the flower replenishing resources (Corbet, 2003). In addition, the 24-hour secretion rate of nectar was calculated by securing PVC mesh bags to flowers with wire ties the day before expected anthesis. Bags had a mesh size of 0.2mm, designed to be permeable to wind and rain yet exclude any pollinators (Corbet, 2003). Bags were then removed around 11:00 h and all nectar extracted individually from 40 staminate and 40 pistillate flowers, over four days (10 staminate and 10 pistillate flowers per day).

Nectar volume (µl) was measured using glass microcapillary tubes (sizes 2, 5, 10 and 20 µl microcaps, Drummond Scientific, Broomall PA, USA) and nectar sugar concentration (mg/mg) was measured using a hand-held refractometer modified for small volumes (Eclipse, Bellingham & Stanley, Tunbridge Wells, UK). Nectar sugar concentration as measured by the refractometer, i.e. weight of solute per weight of solution (*C*; mg/mg), was converted to nectar sugar concentration in terms of weight of solute per volume of solution (*d*; mg/µl) using $d = (0.0037291C + 0.0000178C^2 + 0.9988603)$ (from Prŷs-Jones & Corbet, 1991). The weight of sugar produced per flower over 24 h (*w*) (mg) was then calculated using $w = dvc$, where *v* is volume of nectar (µl) and *c* is sugar concentration of nectar as a proportion (mg/µl).

The amount of pollen (mg per flower) available at a given time point was quantified from 20 stamens which were removed from staminate flowers every 90 minutes from 05:30 to 12:00 h over two days (10 stamens per time point per day) to show pollen depletion within a day. In addition, the total amount of pollen produced in 24 hours was quantified from 40 stamens which were removed from staminate flowers (secured with PVC mesh bags the day before expected anthesis) over two days (20 stamens per day). All stamens were placed in centrifuge tubes in the field.

Owing to the vast quantities of pollen on stamens, pollen was weighed rather than counted. This was done by adding 1ml of distilled water, using a pipette, to centrifuge tubes containing stamens, agitating them for 10 seconds at 12,000 rpm, removing stamens with forceps, and centrifuging at 12,000 rpm for 10 minutes. The supernatant was then removed using a pipette and the centrifuge tube placed in a drying cabinet at approximately 40 °C for 24 hours. The

samples and centrifuge tubes were weighed on a balance before being washed and returned to the drying cabinet for a further 24 hours and weighed again. The weight of the empty centrifuge tube was then subtracted from the centrifuge tube containing the pollen to provide the weight of pollen per flower (mg per flower).

Bee visitation to courgette and wild flowers (2016 and 2017)

To quantify *B. terrestris* abundance at courgette flowers and therefore, their potential pollination efficiency (Figure 5.1), four 50 m transects were established within the crop (from the edge of the crop to the centre, 25m apart). Transects were walked at a steady pace (~5 minutes each) with observations made 1 m either side and in front of the recorder (over a length of 50 m). This was done three times during the blooming period for each site in 2016 and 2017, resulting in a total of 228 transects surveyed over the two years (including data from Cornwall sites in Chapter 4).

In 2017, additional transects in the crop and the field margins were simultaneously surveyed by two observers from 08:15 to 15:30 h at ten sites, resulting in an additional 640 transects. This was to capture pollinator activity in the four hours either side of courgette senescence, which occurs around 12:00 h.

All bee species and the plant species they were feeding on, for nectar or pollen, were recorded to species level. However, *B. terrestris* and bees belonging to the *Bombus lucorum* L. complex were all recorded as "*B. terrestris*" due to difficulties in reliably distinguishing workers in the field (Murray *et al.*, 2008). Sampling was conducted between 08:00 and 10:00 h (when flowers were open) on sunny to partly cloudy days. Since colonies of *B. terrestris* were added to all

fields in 2017, foragers from these colonies are highly likely to have been recorded on pollinator transects.

Pollination of courgette flowers by B. terrestris (2017)

Swabbing B. terrestris for pollen grains

To quantify the number of courgette pollen grains carried on *B. terrestris* and therefore, their potential pollination efficiency (Figure 5.1), *B. terrestris* (n = 17) and *Apis mellifera* (n = 4) were randomly collected from courgette flowers and placed in sample pots. Bees were gently cooled under ice packs, and their entire body swabbed with small cubes of glycerine jelly (with fuchsin dye) positioned on the end of cocktail sticks, before they were released. In the laboratory, microscope slides were prepared by melting the piece of glycerine jelly under a cover slip. The numbers of pollen grains were then calculated under a 20 X magnification (Kremen, Williams and Thorp, 2002).

Pollen grains on stigmas

To quantify courgette pollination, pollen accumulation per stigma was quantified (Figure 5.1). 20 stigmas were removed from pistillate flowers and placed into centrifuge tubes every 90 minutes from 05:30 to 12:00 h over two days (10 stigmas per time point per day). In the laboratory 1/6 of the stigma (one half of a lobe) was dissected and gently squashed onto a microscope slide; fuchsin jelly was then melted over the stigma, under a cover slip (Kremen, Williams and Thorp, 2002). The number of grains were then counted with a 20 X magnification and multiplied by six to achieve an estimate of pollen deposition for the whole stigma.

Yield

To further quantify courgette pollination, yield measurements were also taken (Figure 5.1). To do this, commercial colonies of *B. terrestris* were closed at one field site to quantify courgette yield without managed *B. terrestris* present. This was done over 5 non-consecutive days for a total of 100 pistillate flowers (20 flowers per day), following the methodology for 'open pollination' in Knapp & Osborne (2017) (Chapter 3).

Effect of courgette on B. terrestris colony development (2017)

Pollen loads from B. terrestris

To quantify the proportion of courgette pollen in *B. terrestris*' diet (Figure 5.1), 'forager trap modules' (Martin *et al.*, 2006) were placed onto all commercial colonies within a field for around 45 minutes (between 07:00 and 09:00 h). Once trapped on returning from a foraging trip, workers were narcotised *in situ* using CO_2 for 30 seconds and the number of bees carrying (and not carrying) pollen loads were recorded. One pollen pellet from one of the corbiculae on each bee, i.e. half of their total pollen load, was placed into a centrifuge tube and taken back to the laboratory. Here all pollen loads were sorted to colour and all yellow pollen loads checked to see if they were from courgette, which has large (180 - 200 µm in diameter) and distinctive pollen grains (Nepi, Massimo and Pacini, 1993). A subset (n=56) of all pollen loads were identified to species (where possible) using Sawyer (1981) and a microscope. All foragers were returned to their colony within an hour of being caught and the pollen loads of bees in each field were surveyed on separate days.

Habitat maps

Habitat maps for each study site were required to estimate the amount of forage and nesting sites (semi-natural habitat and mass-flowering crops) available to bumblebees in the landscape (Figure 5.1) (Westphal, Steffan-Dewenter and Tscharntke, 2003; Kremen *et al.*, 2004). To create these maps, semi-natural habitat (woodlands and heathland), improved grassland, and mass-flowering crops (courgette and maize) were recorded in 750m radii of each field site in 2017 (n = 14). This was done by ground truthing satellite imagery and adapting Land Cover 2007 data (Centre for Ecology and Hydrology 2011) using ArcGIS 10.2.2 (Figure 5.2). Each site had varying quantities of crop and habitat types (Figure A 4.1).

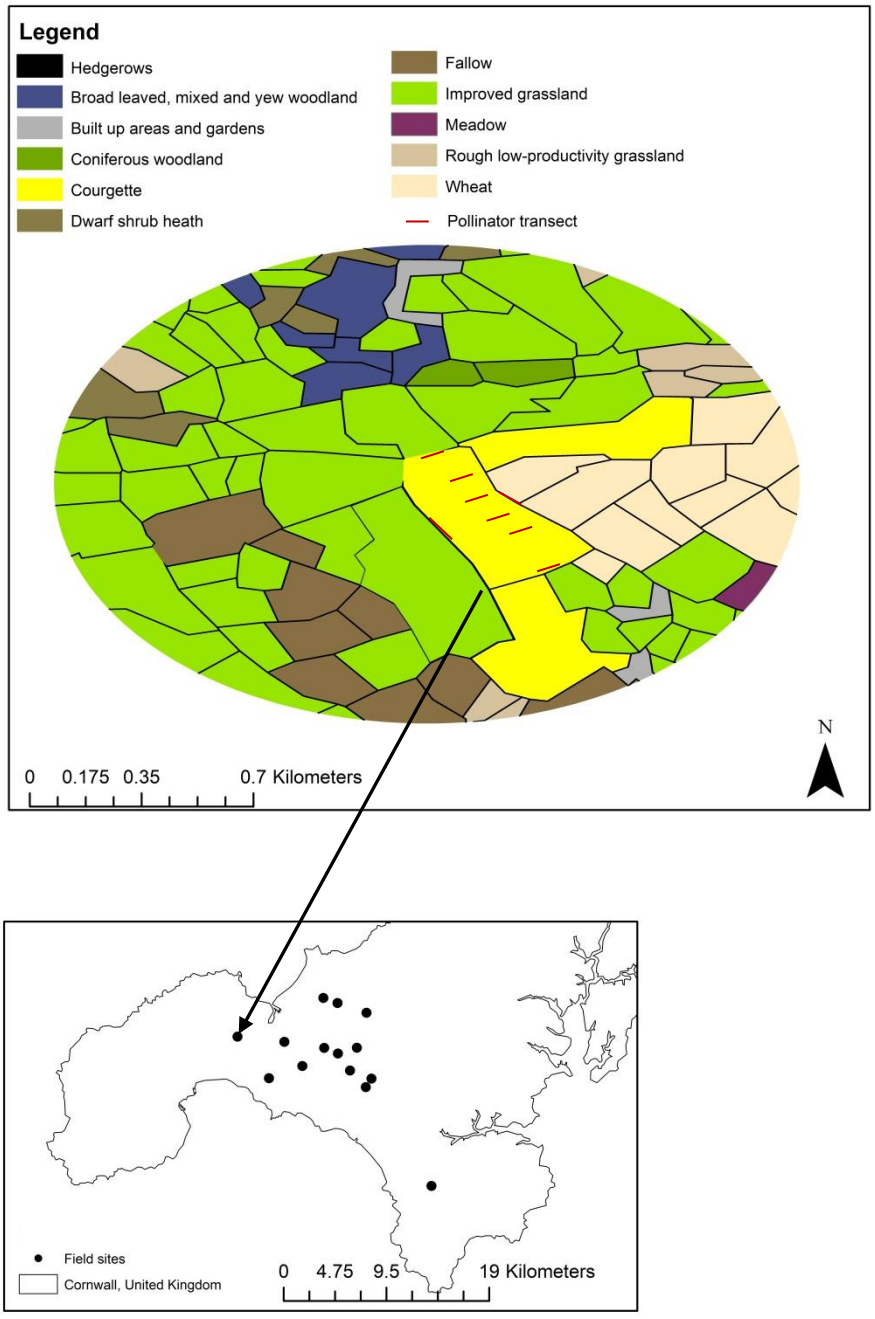

Figure 5.2 Map of a landscape surrounding a study site in Cornwall, UK (including pollinator transects within a courgette field). All study sites surveyed in 2017 (Cornwall, UK) are also shown.

Bumble-BEEHAVE simulations using BEE-STEWARD

The default settings for *Bumble*-BEEHAVE provide on the 1st of January simulations with 500 queens who randomly emerge from hibernation on 1st of April (± 28 days *SD*), following a normal distribution. In the model, queens can nest in all types of semi-natural habitat implemented in the model: heathland,

species-rich grassland, hedgerow, scrub and woodland (Becher, Twiston-Davies *et al.* 2018).

Habitat types are defined by the presence and abundance of 44 forage plants which provide nectar and or pollen during specified flowering periods. Once a queen has found suitable nesting habitat she must collect sufficient pollen and nectar resources before laying her first batch of eggs. She will then continue to split her time between foraging and brood care until the first adult workers emerge. The queen will then focus on egg-laying whilst workers divide their time between brood-care and foraging. Foraging choices are based on maximising foraging rate (pollen) or energetic efficiency (nectar), which depends on distance, handling time, and the degree of patch depletion. The probability of a bee detecting a new patch is based on the distance of the food source from a colony. Towards the end of colony development female larvae may develop into queens, and the original queen switches from laying diploid eggs to haploid, male eggs. Once new queens are developed they leave their colony, mate and hibernate prior to emergence the following year. For a detailed model description see supplementary material S03 (‘ODD protocol’) of Becher & Twiston-Davies *et al.* (2018).

BEE-STEWARD's flexible input settings meant that habitat types recorded on surveys, which were not already in the model (i.e. courgette, heathland, and improved grassland) could be easily parametrised in the input files for analysis (Table A 4.1). Courgette fields were specified as either ‘early courgette’ (flowering from the beginning of June until the middle of July) or ‘late courgette’ (flowering from the middle of July until the end of August) to reflect the cropping practices of courgette production in the UK. A map of each study site was

separately input into the model and manually edited (if needed) using the functions available within the program (Becher et al., 2016).

In order to reduce computational time and to ensure that simulations were based solely on populations in equilibrium (Hui, 2006), a set of preliminary simulations were run in landscapes with no courgette (where courgette fields had been temporarily removed) as a baseline. To determine a suitable number of initial queens for all landscapes, simulations were started with 500 hibernating queens and run over 15 years in each landscape 20 times. The number of queens was then plotted over time to see at what number of queens the population appeared to reach equilibrium (Figure A 4.2). This resulted in 500 hibernating queens as a conservative estimate for all landscapes and simulations. To determine the length of simulations (i.e. time taken to reach equilibrium), simulations were run starting with a population size that was either close to the estimated number of hibernating queens (500) or above it (1000) across all landscapes (with no courgette) 20 times, over 20 years. The population was assumed to be in equilibrium, once both growth curves had converged (Figure A 4.3). Year 11 was taken as the year where all landscapes were in equilibrium.

The effect of courgette on *B. terrestris* population dynamics was explored by re-classifying courgette fields in landscape maps of actual study sites to either ‘early season courgette’, ‘late season courgette’ or ‘no courgette’ in BEE-STEWARD. This created three different cropping scenarios for simulations in *Bumble*-BEEHAVE: 1) no mass-flowering crop (baseline), 2) early season courgette, and 3) late season courgette (Table A 4.1). All simulations were run 10 times per landscape and cropping scenario, totalling 420 simulations.

The average number of over-wintering queens, colonies, and adult workers, as well as the nectar and pollen stores per colony, were calculated daily for each landscape over 11 years.

Statistical analysis

All analyses were carried out using R (R Core Team, 2017). For empirical data, independent sample *t*-tests were used to compare the differences in mean sugar production (g) between staminate and pistillate flowers (over 24 hours and every 90 minutes), pollen depletion (mg/flower) between 05:30 and 10:00 h, pollen accumulation on stigmas (grains/stigma) between 05:30 and 11:30 h., and *B. terrestris* abundance in the margin and cropped area per hour.

For simulated data the effect of cropping scenario (fixed effect) on the numbers of hibernating queens (day 365), colonies (day 149), adult workers (day 149), and adult workers per colony (day 235) in year 11 was tested using linear models. *Post hoc* Tukey tests were calculated using the multcomp package (Hothorn, Bretz and Westfall, 2008). All means are presented with their associated standard error unless otherwise stated.

Results

Nectar and pollen measurements from courgette

The secretion rate of nectar, i.e. the weight of sugar produced over 24 hours (from bagged flowers) was greater (although not statistically, T_{78} = -1.94, P = 0.06) for pistillate flowers (34.41 ± 2.67 mg per flower, n = 40) than staminate flowers (26.59 ± 1.56 mg per flower, n = 40). These estimates were much higher than the nectar standing crop, i.e. weight of sugar available at a given time point per flower, which at 05:30 h was just 0.52 ± 0.09 mg for pistillate flowers and 1.24 ± 0.16 mg for staminate flowers (Figure 5.3a). By 11:30 h

nearly all sugar was depleted from both staminate (0.05 ± 0.01 mg) and pistillate (0.07 ± 0.01 mg) flowers (Figure 5.3a).

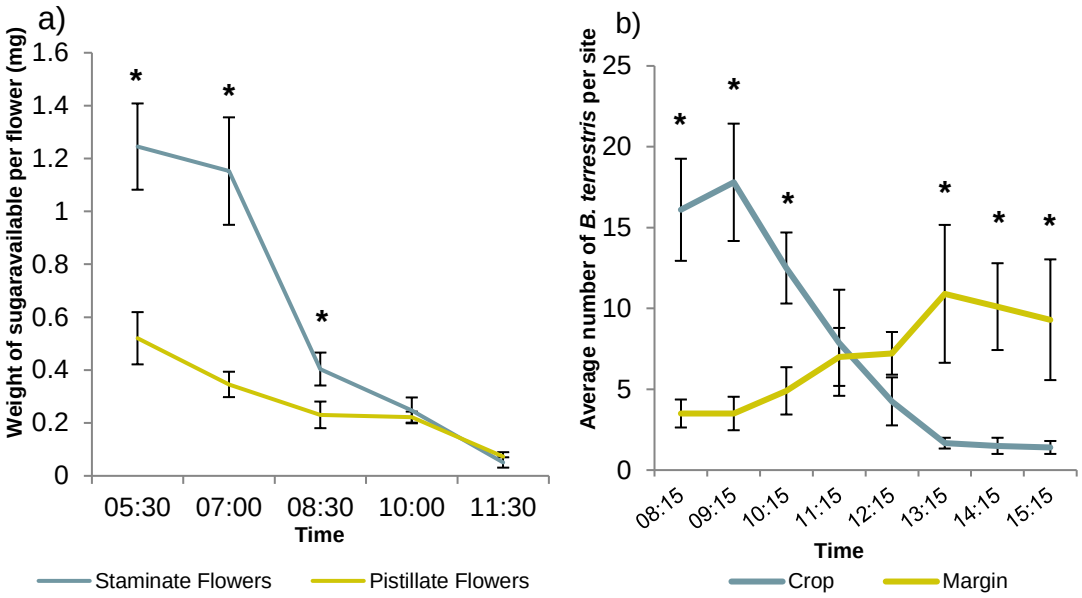

Figure 5.3 a) Weight of sugar available (± *SE*) every 90 minutes for 40 staminate and 40 pistillate flowers (400 flowers in total) and 2b) average number of *B. terrestris* in the crop and on the margin over time, data were summed per transect in either the crop or on the margin and averaged by site (n = 10), all sites contained commercial colonies of *B. terrestris*. Significant independent *t*-tests are indicated with an asterisk (*) for each time point ($P < 0.05$).

The weight of pollen produced over 24 hours (from bagged flowers) was 18.04 ± 0.84 mg per staminate flower (n= 40). Again, this was much greater than the weight of pollen available from un-bagged flowers, which was estimated to be 10.96 ± 1.39 mg per flower at 05:30 h. From 05:30 to 10:00 h there was no significant loss (T_{37} = -1.22, P = 0.23) of pollen (10:00 h = 8.37 ± 1.64 mg per flower) suggesting that much of the pollen is removed around anthesis when the very first pollinator visits occur.

Visitation to courgette and wild flowers

A. mellifera and *B. terrestris* were the most abundant pollinator species observed visiting courgette flowers across the two years of this study (although commercial colonies of *B. terrestris* were added to fields in 2017) (Figure 5.4). *B. terrestris* showed equal preference to staminate and pistillate flowers (Figure 5.4) which may affect pollination efficiency.

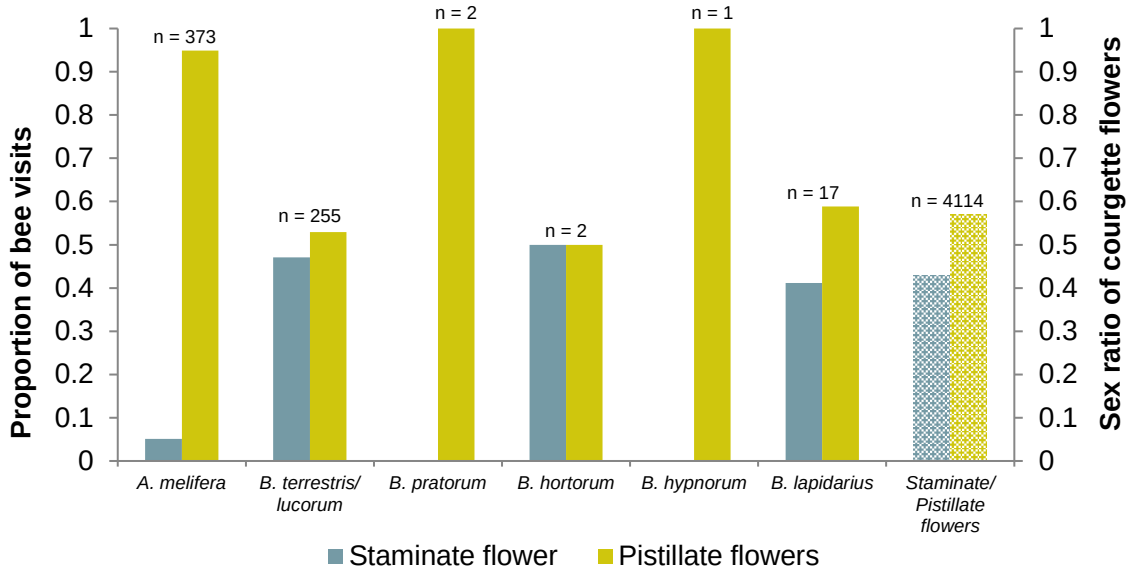

Figure 5.4 Proportion of nectar visits to staminate and pistillate flowers for *A. mellifera*, *B. terrestris/ lucorum*, *B. pratorum*, *B. hypnorum* and *B. lapidarius* recorded on pollinator transects in 2016 and 2017, as well as the proportion of staminate and pistillate flowers on floral transects in 2016 and 2017. Data were pooled from all transects conducted in the cropped area of 19 fields.

In the morning, *B. terrestris* was significantly more abundant in the crop when courgette flowers were open and providing nectar, than in the margin (Figure 5.3b). However, in the afternoon *B. terrestris* were significantly more abundant in the margin than in the crop when courgette flowers are closed and no longer providing nectar (Figure 5.3b).

Pollination of courgette flowers

B. terrestris carried an average of 1866 ± 476 (n = 13) pollen grains on their bodies, more than *A. mellifera* which carried an average of 122 ± 39 (n = 4) pollen grains on their bodies.

By 11:30 h an average of 4749 ± 441 (n = 18) pollen grains had been deposited onto stigmas, significantly more (T_{32} = -5.52, P = <0.001) than at 05:30 h 1879 ± 276 (n = 16).

The percentage of open-pollinated pistillate flowers setting fruit was very high across the 5 days of surveying at 97 ± 2% (n = 96).

Effect of courgette on B. terrestris colony development

Pollen loads

None of the 394 pollen loads collected from *B. terrestris* contained courgette pollen (Table A 4.2). Brassica spp. (15), bramble (11), and common poppy (7) were the most common pollen species identified out of a subsample (n = 56) of pollen loads (Table A 4.2). Consequently, all courgette flowers were specified as having a pollen resource value of zero in BEE-STEWARD (Table A 4.1).

Bumble-BEEHAVE simulations using BEE-STEWARD

Early courgette landscapes had a higher 'carrying capacity' for queen bumblebees, determined by the number of over-wintering queens on the last day of the year compared to no courgette (contrast estimate -419.0 ± 157.9, T = 2.65, P = 0.03) and late courgette (contrast estimate -435.7 ± 157.9, T = 2.76, P = 0.02) on day 365 (Figure 5.5). Likewise early courgette resulted in the establishment of more colonies in the landscape compared to no courgette (contrast estimate -30.53 ± 11.43, T = -2.67, P = 0.03), and late season

courgette (contrast estimate -31.34 ± 11.43, *T* = -2.74, *P* = 0.03) on day 149 (day with the largest differences between cropping scenarios) (Figure 5.6). This resulted in more adult workers on day 149 (peak forager activity) across early courgette landscapes compared to no courgette (contrast estimate -474.86 ± 189.6, *T* = -2.51, *P* = 0.04) and late courgette (contrast estimate -534.29 ± 189.6, *T* = -2.82, *P* = 0.02) landscapes (Figure 5.7). Indeed, the year on year effect of early courgette also increased the abundance of foragers early in the season, before courgette flowering (Figure 5.7). More adult workers in early courgette landscapes meant that more resources in the wider landscapes were utilised, thus there were more nectar visits to semi-natural habitat in landscapes with early courgette compared to late, and no courgette landscapes even though all cropping scenarios had the same amount of semi-natural habitat (Figure A 4.4). Whilst early courgette landscapes lead to the establishment of more colonies, the average size of each colony did not increase compared to no courgette (contrast estimate -7.13 ± 6.44, *T* = 1.08, *P* = 0.5) and late courgette (contrast estimate -0.16 ± 6.44, *T* = -0.02, *P* = 1.00) on day 149 (peak forager activity) (Figure 5.8~~Figure 5.8~~). The availability of nectar or pollen per bee was also similar between cropping scenarios (Figure 5.9~~Figure 5.9~~a and 5.9b). Since pollen and nectar stores per bee were only calculated on days where colonies had more than 100 adult workers (to avoid too many peaks in stores when populations were low), early courgette landscapes appear to have more pollen and nectar available per bee at the beginning and end of the season (Figure 5.9~~Figure 5.9~~a and 5.9b), however, this is more likely due to more adult workers in these landscapes (Figure 5.7). The phenology of early season courgette (flowering from beginning of June to the middle of July) is more closely related to forager activity (indicated with the baseline, no courgette) and

longer in duration than late season courgette (flowering from middle of July until the end of August) (Figure 5.7).

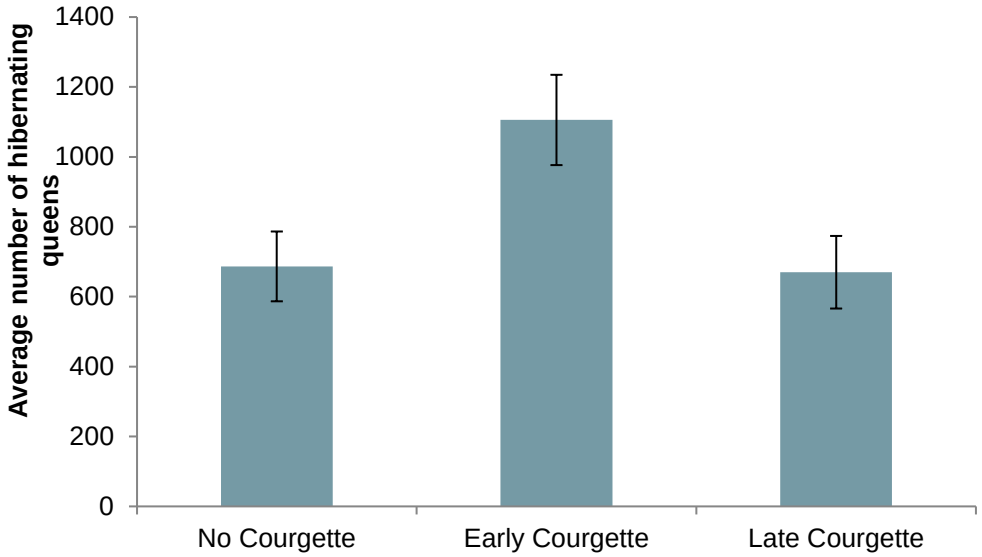

Figure 5.5 Average number of hibernating queens ± *SE* on the last day of year (year 11) for each cropping scenario. Data were averaged across the 10 repeated runs and 14 study sites.

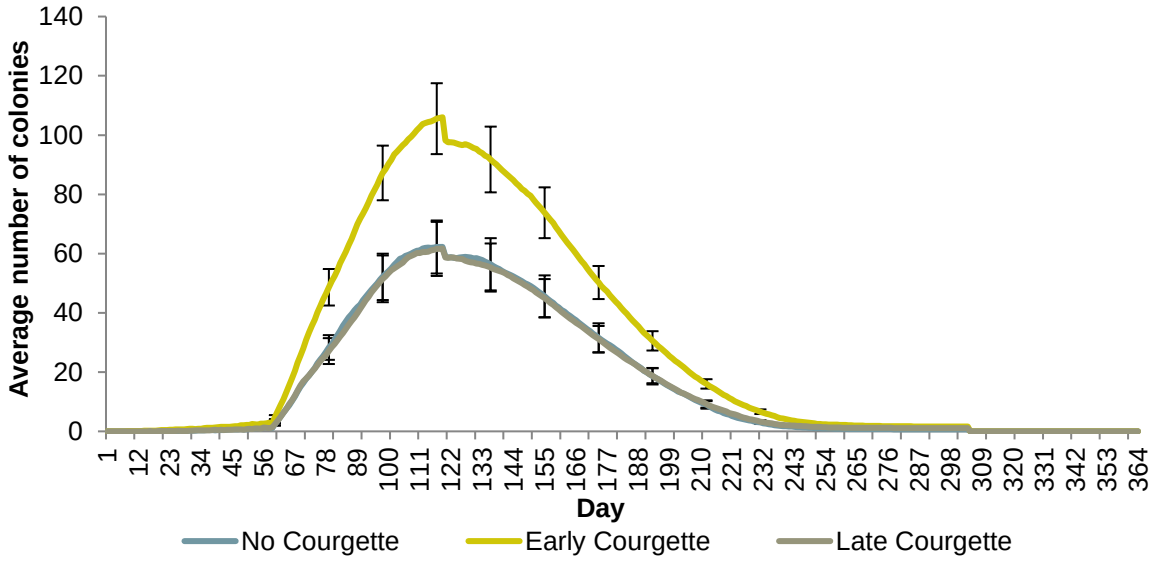

Figure 5.6 Average number of colonies (± *SE* every 20 days) over the course of a year (year 11) for each cropping scenario. Data were averaged across the 10 repeated runs and 14 study sites.

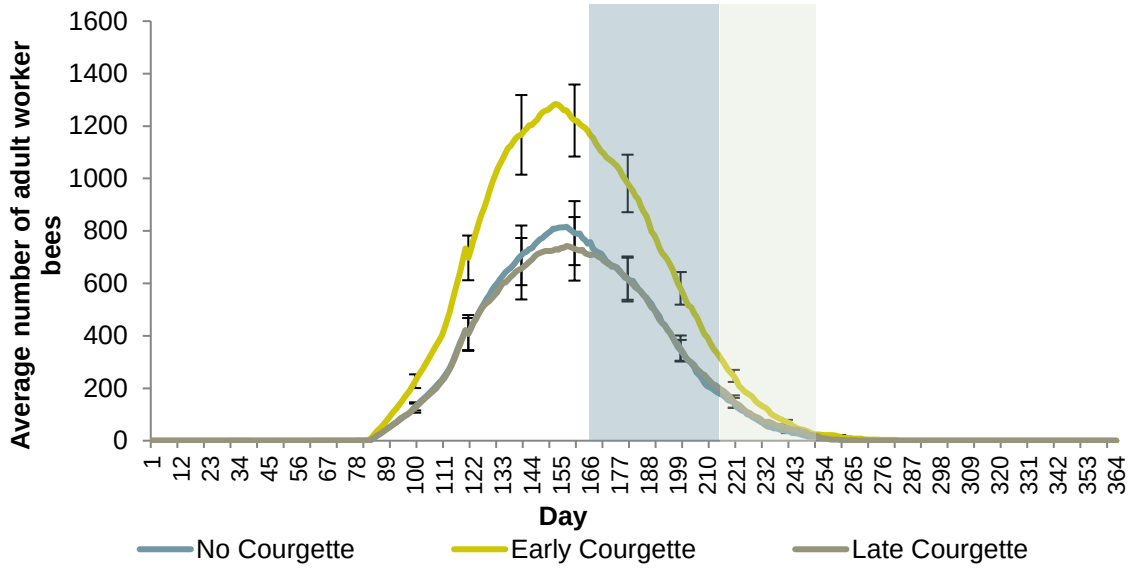

Figure 5.7 Average number of adult worker bees (± *SE* every 20 days) over the course of a year (year 11) for each cropping scenario. Shaded areas show the flowering times of courgette, early courgette is shown in dark grey, late courgette shown in light grey. Data were averaged across the 10 repeated runs and 14 study sites.

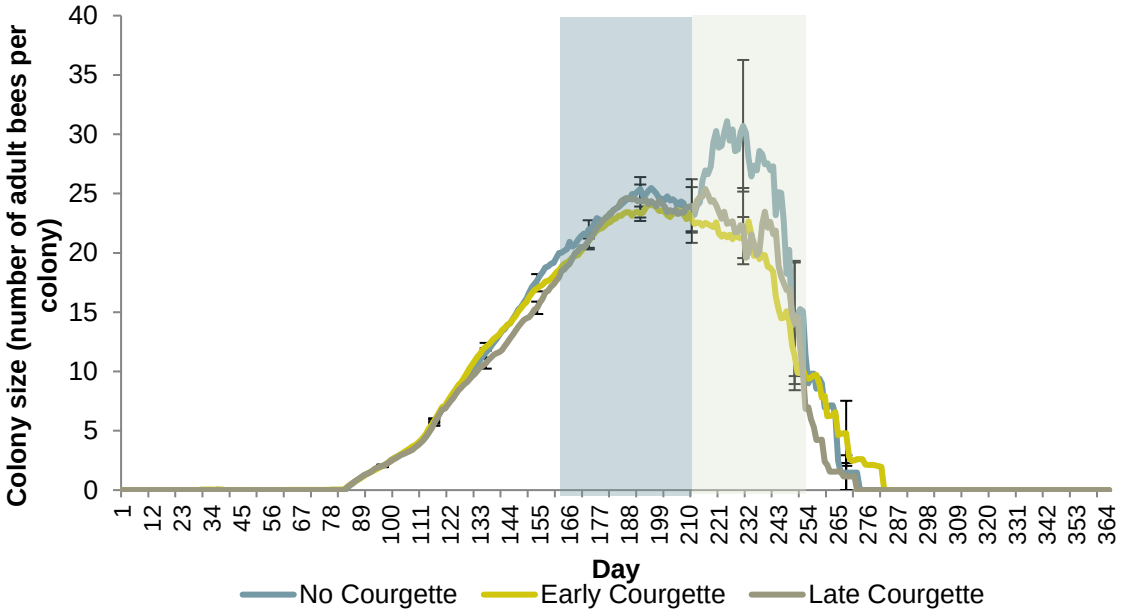

Figure 5.8 Average size of colonies (number of adult bees per colony) (± *SE* every 20 days) over the course of a year (year 11) for each cropping scenario. Shaded areas show the flowering times of courgette, early courgette is shown in dark grey, late courgette shown in light grey. Data were averaged across the 10 repeated runs and 14 study sites

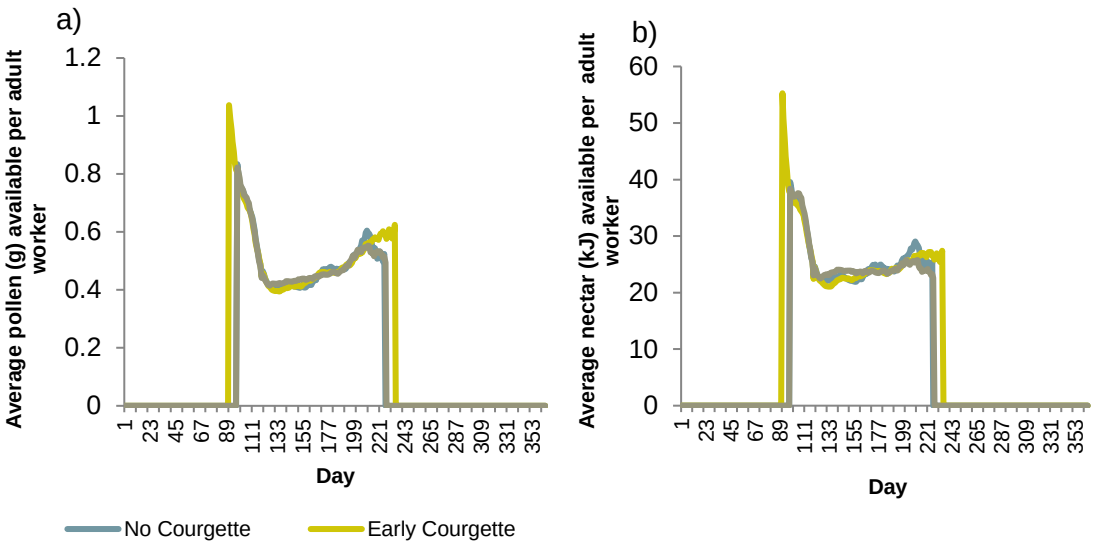

Figure 5.9 a) pollen (g), and b) nectar (kJ) available per adult worker for each cropping scenario in year 11, n.b. early courgette flowers for longer than late courgette. The total average pollen and nectar stores were divided by the total average number of adult workers, although only when colonies had more than 100 adult workers to avoid too many peaks in stores when populations were low. Data were averaged across the 10 repeated runs and 14 study sites.

Discussion

This study clearly demonstrates a mutualistic interaction between courgette flowers and *B. terrestris* that is beneficial to both, improving pollination success and colony dynamics (Bailes *et al.* 2015; Holzschuh *et al.* 2016). Courgette, like many other mass-flowering crops offers vast quantities of nectar and pollen to attract pollinators to its flowers for pollination (Vidal *et al.*, 2006). Indeed per m^2, courgette offers more nectar (0.35 ml) than oilseed rape (0.30 ml), field bean (0.092 ml) and sunflower (0.003 ml) (Becher *et al.*, 2016), and is therefore a high value mass-flowering crop in terms of nectar production.

Results showed that over 24 hours pistillate flowers produce significantly more sugar than staminate flowers. However, when measured every 90 minutes staminate flowers offer more sugar than pistillate flowers. The overall higher sugar content combined with harder to access nectaries (Nepi, Massimo and

Pacini, 1993) means that bee species can have longer handling times and a preference for pistillate flowers (Tepedino, 1981; Artz and Nault, 2011; Phillips and Gardiner, 2015). In this study, *B. terrestris* chose staminate and pistillate courgette flowers more equally than *A. mellifera* which is desirable for optimum pollen transfer (Figure 5.4, see also Figure 4.2~~Figure 4.2~~, Chapter 4). At a field scale, *B. terrestris* also showed a strong fidelity to courgette, visiting crop flowers more often than wild flowers in the hedgerows, in the morning when courgette flowers were open, providing the first empirical evidence of *B. terrestris* fidelity to a *Cucurbita* crop (Petersen, Reiners & Nault 2013).

In this study, the majority of pollen was removed around anthesis during the very first pollinator visits (Stanghellini, Schultheis and Ambrose, 2002; Phillips and Gardiner, 2015). However, personal observations showed *B. terrestris* removing excess pollen grains from their bodies early in the morning (Figure A 4.5), supporting the findings of Nepi, Massimo & Pacini (1993)) despite courgette pollen having a high crude protein content (38.6%) (Petersen, Reiners and Nault, 2013). Nonetheless, *B. terrestris* was still observed to carry more loose pollen grains on their body and therefore, have a higher pollination potential than *A. mellifera*. Indeed, pollen was still transferred to stigmas well after anthesis, shown by significantly more pollen grains on stigmas at the end compared to the beginning of the morning. By the end of the morning stigmas had received an adequate number of pollen grains (4749 ± 441) for optimum fruit set (~1200 required for maximal fruit set in pumpkin (Vidal *et al.* 2010)), as evidenced by the high percentage fruit set and therefore, very low pollination deficit in this study (see also Chapter 3). Despite courgette pollen being relatively high in protein (Petersen, Reiners & Nault 2013), its large sticky grains may make it difficult for *B. terrestris* to collect (Vaissière & Vinson 1994).

Therefore, *B. terrestris* may avoid collecting *Cucurbita* pollen, since as a generalist species it can visit alternative, more easily obtainable pollen, unlike *Peponapis* and *Xenoglossa* spp. which as *Cucurbita* specialists are thought to rear their offspring exclusively on *Cucurbita* pollen (Tepedino 1981, Chapter 1). This may be why no pollen loads from returning *B. terrestris* foragers contained courgette pollen.

After courgette flower senescence (within a day) *B. terrestris* appeared to 'switch' from courgette to hedgerow flowers, evidenced by the diverse range of pollen loads collected from returning *B. terrestris* foragers. Whilst some of the plant species from pollen loads may occur in hedgerows immediately surrounding courgette fields, others may be from species located further away. This highlights the importance of maintaining wild flowers at different spatial scales to fulfil bees' requirements for nectar and pollen beyond that of the focal crop. Indeed flower rich areas have been shown to increase colony density (Wood *et al.*, 2015) and food supplementation shown to increase colony development, particularly of queen and male bumblebees (Pelletier and McNeil, 2003). However, the extent to which pollinators are attracted into mass-flowering crops will vary depending on the relative quality and quantity of floral resources in the mass-flowering crop and nearby semi-natural habitat. In this study it appears that providing additional floral resources to mass-flowering courgette facilitates pollination services to courgette, supporting bumblebee nutrition without distracting bees from courgette flowers. Indeed, wild flower species richness in courgette fields have been shown to be the most important factor for determining bumblebee abundance at courgette flowers and could therefore be used to attract bumblebees into courgette fields whilst providing additional forage (Chapter 4).

5. Bombus terrestris and courgette: A mutualistic relationship?

Given courgette's bountiful, yet transient supply of nectar, bumblebee population dynamics were shown (using *Bumble*-BEEHAVE) to improve in landscapes with early flowering courgette compared to a no courgette baseline. As bumblebee foragers are generally most active mid-summer, early courgette was the best cropping scenario for concurrently achieving more forager visits (pollination potential) and more food (nectar only) to be brought back to the colony. Whilst courgette nectar provides additional energy, helping to reduce foraging efforts, bees can only benefit if protein providing pollen is also available to raise their brood. Empirical data showed that, within a day, bees were able to utilise courgette for nectar *and* wild flowers for pollen (Figure 5.3b), thus supporting model results which showed (at a coarser temporal scale) that with more nectar, colonies were able to grow and subsequently forage on more, additional resources for pollen. Subsequently early courgette supports more adult workers (foragers), colonies, and hibernating queens for subsequent years compared to late, and no courgette landscapes. Nonetheless, planting early courgette and late courgette in fields adjacent to each other could improve forager numbers in late courgette and further improve bumblebee populations for subsequent years (Riedinger *et al.*, 2014).

The phenological matching of crops with key periods of pollinator activity is thought to be why the presence of oilseed rape in the landscape (early in the season) can improve the reproductive potential of *Osmia bicornis* L. (Jauker *et al.*, 2012; Holzschuh *et al.*, 2013), but not *Bombus pascuorum* S. (Herrmann *et al.*, 2007) and *B. terrestris* (Westphal, Steffan-Dewenter and Tscharntke, 2009). This is because whilst oilseed rape can improve colony establishment and growth of bumblebees, the lack of resources later in the season mean there is

no increase in the number of males or queens produced (Herrmann *et al.*, 2007; Westphal, Steffan-Dewenter and Tscharntke, 2009).

This lack of phenological matching is also true of late courgette which despite offering resources later in the season (unlike oilseed rape) still misses the key period of bumblebee foraging. However, Rundlöf *et al.* (2014) observed more queen and male bumblebees on transects around fields of late-flowering red clover, suggesting results could be specific to flower and pollinator species. Since nectar and pollen supplies in the landscape are directly influenced by colony density, it is perhaps unsurprising that colonies in early courgette landscapes had similar amounts of pollen and nectar available per bee compared to late and no courgette landscapes. Interestingly, the average pollen and nectar store per bee and average number of colonies per landscape decline around day 116, which may be a result of willow species (common to hedgerows and scrub in *Bumble*-BEEHAVE's input files) no longer flowering.

Conclusion

Combining empirical data on pollinator visitation, nectar and pollen availability, and pollination efficiency, with model simulations has provided a unique insight into the mutualistic relationship between *B. terrestris* and the mass-flowering crop, courgette. Flower-scale data (within a day) showed how effective a pollinator *B. terrestris* is in courgette and the extent to which they utilise courgette flowers for pollen and nectar. Based on this information *Bumble*-BEEHAVE was parameterised to show the effect of courgette management at the crop-scale (within a year) which, whilst theoretical, is consistent with empirical knowledge.

Broadly, these findings show that matching crop phenology with key periods of forager activity can be an effective way of improving bumblebee population dynamics and pollination efficiency. Increased understanding of a plant-pollinator mutualism at different temporal and spatial scales means that management recommendations can be made. For growers this may mean planting mass-flowering crops with complementary phenologies (such as early and late courgette) in fields adjacent to each other. For conservationists it may mean recognising the importance of courgette, alongside other mass-flowering crops, as valuable forage resources for bumblebees, whilst continuing to promote additional sources of forage to fulfil bees' nutritional requirements over space and time. In doing so, it could be possible to simultaneously improve pollination services *and* bumblebee populations in intensive farmland.

6. Thesis discussion

Plate 9 Staminate courgette flower. Photograph by Daphne Wong.

General discussion

Each data chapter of this thesis has drawn on different techniques and methodologies to determine the extent to which pollination influences fruit set (the mechanism), how pollination could be improved (the management), and how in doing so, growers' profits and agricultural resilience could be increased (the outcomes) (Figure 6.1). Where 'agricultural resilience' refers to a grower's ability to produce sufficient, nutritious food during periods of adverse environmental conditions, and therefore stable yields over time (Bullock *et al.*, 2017).

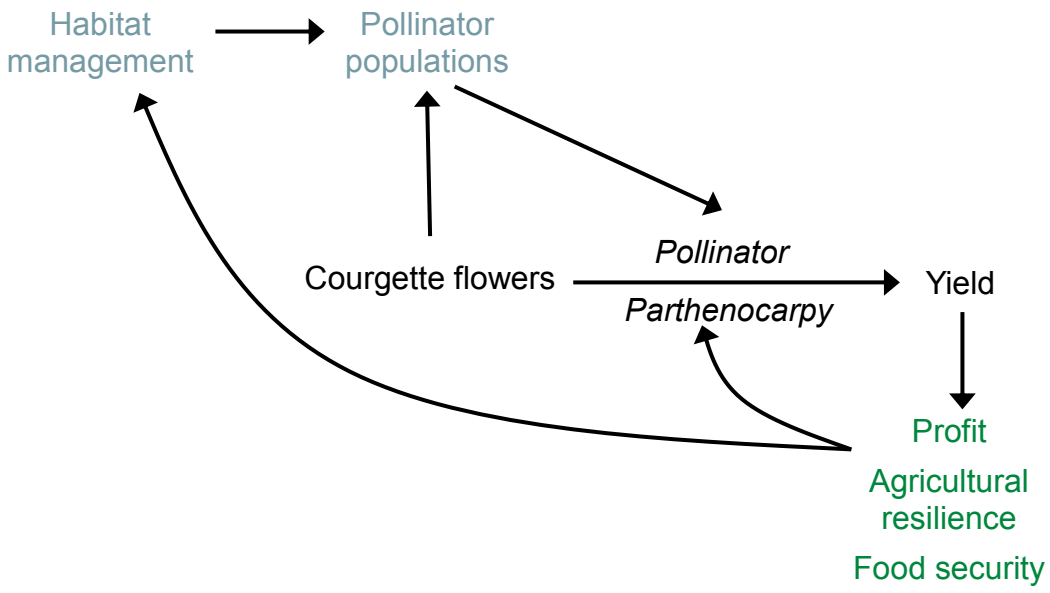

Figure 6.1 Mechanisms of (black text), management for (blue text), and outcomes of (green text) cucurbit pollination, investigated in this thesis using UK field-grown courgettes as a model system.

Can courgette pollination be improved?

Pollination experiments in the courgette variety 'Tosca' showed that insect pollination increased yield by 39% and that there was no evidence of pollination

limitation on crop yield, evidenced by a low pollination deficit of just 3% (Chapter 3). Unexpectedly, 56% of fruit was able to reach marketable size and shape without any pollination, resulting in further experiments in a controlled temperature room with no pollinating insects on three additional varieties: ‘Parthenon’ (selectively bred to be parthenocarpic but not currently used by commercial growers), ‘Greco’ and ‘Tosca’, to compare to field, no-pollination experiments (Plate 10). However, after two attempts this experiment was ended as it was difficult to ensure that soil was not contaminated with insects and that plants were healthy enough to not affect fruit set. Growing different varieties in the CT room was of particular interest given that plastic pollination bags (used in Chapter 3) may elevate levels of ethylene and therefore influence fruit set. Likewise, different varieties are likely to vary in their level of pollinator dependence. Nonetheless, evidence of parthenocarpy is rarely discussed in agroecology and inspired Chapter 2 to quantify the extent and effectiveness of parthenocarpy in other crop species, which like courgette are also considered to be ‘pollinator-dependent’.

Plate 10 CT room experiment exploring the pollination requirements of three courgette varieties ‘Parthenon’ (selectively bred parthenocarpic variety, not currently used by commercial growers), ‘Greco’, and ‘Tosca’.

Whilst the yield of ‘Tosca’ was less dependent on pollination than expected, the total economic value of insect pollination to courgette production was still estimated to be worth approximately £3,398/ha. However, this economic valuation was based on the pollinator dependency and pollination deficit of just one courgette variety ’Tosca’, thus inter-variety differences in pollinator dependence, or site-specific levels of pollination deficit may increase or decrease this economic value. For example, Garratt *et al.* (2014) estimated the apple variety ‘Cox’ to have a pollination deficit of £146/ha, compared to the variety ‘Gala’ which had a much higher pollination deficit of £6,459/ha. This was due to ‘Gala’ being more pollinator-dependent and having a larger pollination deficit (higher yield from hand-pollinated flowers) compared to ‘Cox’. Whilst economic valuations are based on relatively simple estimates of pollinator dependence, pollination levels, and growing practices (which may not be

representative of a larger spatial scale), they do clearly demonstrate the importance of pollinators to crop production (Gallai *et al.*, 2009).

Since growers may not know the level of pollination and therefore their potential pollination deficit, it would be useful to develop a predictive model which could determine if managed pollinators are required and/ or if longer term pollinator habitat creation is required. Although other pollination service models exist (e.g. Olsson *et al.* 2015), none have the capabilities of BEE-STEWARD (www.beehave-model.net) which has an interface which already enables users to simulate the effects that different management options, such as wild flower strips will have on forager numbers and therefore, pollination potential. Thus, BEE-STEWARD would only need to be parametrised with crop/ variety's pollination requirements for bespoke simulations to be run for growers based on their level of pollinator dependence and likely abundances of bees (based on landscape maps).

***How* can courgette pollination be improved?**

Whilst courgette is grown at different sites each year, *A. mellifera* and *B. terrestris* were the most abundant pollinators of courgette, occurring in their greatest numbers within the cropped area, at all field sites over the three field seasons of this thesis. Whilst both species show a preference for courgette flowers, Chapters 4 and 5 showed *B. terrestris* had a more equal preference for staminate and pistillate courgette flowers and carried more loose pollen grains (desirable for optimum pollen transfer) than *A. mellifera*. These findings also demonstrate that several widespread, resilient species (rather than species-rich communities) can fulfil the pollination requirements of courgette and that in the UK maximal yields can still be achieved without *Peponapis* and *Xenoglossa*

species. A full species list of pollinator species recorded in courgette fields in 2016 and 2017 are in Appendix 3.3.

Although these species respond positively to increased provision of floral resources (Chapter 4) their populations can also be artificially increased by beekeepers and growers through the introduction of managed colonies. However, the degree to which managed pollinator species will improve yield depends on the pollination deficit at a given site. For example, results from the pollinator supplementation experiment (Appendix 5.2) at field sites in Cornwall (3% pollination deficit observed the year prior) showed that a field stocked with managed *B. terrestris* colonies (Biobest Biological Systems, Belgium) at the recommended stocking density for courgette (Koppert Biological Systems, Netherlands) was unable to significantly increase yield. Therefore, the recommendation of Chapter 3; to use managed bumblebee colonies to maximise yields (to hand pollination levels), are likely unfeasible in areas with a very low pollination deficit. Indeed managed colonies of *B. impatiens* were also unable to increase pumpkin yield, owing to already high levels of pollination at study sites in New York State (Petersen, Reiners and Nault, 2013; Petersen, Huseth and Nault, 2014).

Nonetheless, areas with a greater pollination deficit may benefit from using managed pollinator species. For example, Artz, Hsu & Nault (2011) observed a significant increase in pumpkin yield following the addition of managed *B. impatiens* colonies at study sites which were also in New York State. This demonstrates the potential for spatial and temporal fluctuations in wild pollinator communities within a region, which may or may not result in pollination deficits. However, growers should be cautious not to rely on single species to fulfil their

pollination needs, since cucurbits could also experience greater yields in more diverse habitats; where increased species richness and abundance of wild pollinators could improve spatial and temporal fluctuations in pollination services (Hoehn *et al.*, 2008; Garibaldi *et al.*, 2011), provide insurance against any pollinator loss (Shuler, Roulston and Farris, 2005) and reduce the spread of disease and pathogens (Kremen and Miles, 2012). Therefore, promoting effective wild pollinators of courgette, through sustainable pollinator-supportive practices, could be the best way to increase grower's agricultural resilience for pollination services.

To make recommendations on how growers could maintain or promote the high level of pollination observed in Chapter 3, it was necessary to determine the relationship between floral resources (of both courgette and wild flowers) and pollinators in courgette fields. Therefore, following an advert in AHDB's news bulletin for growers of 'Tosca', four more study sites (two in Worcestershire and two in Cambridgeshire) were selected to achieve a natural gradient of pollinators and floral resources at study sites. All sites had naturally occurring wild flowers in and around courgette flowers which provided an excellent opportunity to understand the extent to which floral resources mediate pollinator visitation to courgette flowers and courgette fields, at different spatial scales: field scale (in margins, and in the cropped area) and farm scale (500 m and 2000 m radii) (Chapter 4). Data were also collected at each site on soil pH, nitrogen, phosphorous, and potassium, as well as courgette yield at the end of the season (from farmers), in order to link the positive, mediating effect of forage availability on pollinator visitation and crop yield (although this has previously been shown in pumpkin in the US (Petersen, Jessica and Nault, 2014). However, having single values for yield, per field, over the whole season,

rather than measures of yield which directly followed the survey rounds at each site, meant that the sample size was too small to detect any significant effects (Appendix 3.1). However, the methodology for this experiment is in place should it ever be repeated at a larger spatial scale.

Nonetheless, combining pollinator transects with pan trapping meant that the overall pollinator species richness of courgette fields was better represented, allowing management decisions to be formulated which could improve crop pollination, *or* species conservation; or both (Kleijn *et al.*, 2015). Naturally occurring wild flowers (e.g. agricultural weeds and hedgerow flowers) are frequently overlooked floral resources for pollinators (Bretagnolle and Gaba, 2015) despite being free and sustainable, and thus more likely to be implemented by growers. Understanding the relationship and possible trade-offs between pollination and resource competition between courgette and co-flowering wildflowers was beyond the scope of this thesis (Figure 1.2, Chapter 1). However, growers' concerns are likely to be minimal since many courgettes in the UK are grown in black plastic to supress 'weeds' from growing immediately around crop plants (Plate 11). Indeed, wild flowers within the crop may have the additional advantage for growers of improving soil structure which is vital for courgette fields picked daily using large tractors and rigs, especially during periods of heavy rainfall (Plate 11).

Plate 11 Damage to the soil following a period of heavy rainfall; two tractors were being used in this photograph to tow the rig. The use of black plastic to suppress ‘weed’ growth immediately around courgettes is also shown.

Data from Chapters 4 and 5 did, however, show that wild flowers are unlikely to be competing with crop flowers for pollination services. For example, Chapter 5 showed *B. terrestris* visiting crop flowers more often than wild flowers in the hedgerows, in the morning when courgette flowers were open, before ‘switching’ to hedgerow flowers after courgette senescence, providing the first evidence of *B. terrestris* fidelity to a *Cucurbita* crop (Petersen, Reiners and Nault, 2013). This was also supported by data from Chapter 4 which showed that the abundance of honeybees and bumblebees in field margins did not significantly reduce their abundance on courgette flowers.

Although solitary bees were not observed to visit courgette flowers, their abundance and species richness in courgette fields were significantly greater with more wild flower species and semi-natural habitat surrounding a site. Therefore, allowing uncultivated areas around the crop to be colonised by wild

flowers is an effective way of boosting the abundance of bumblebees - important visitors to courgette flowers, as well as the abundance and species richness of solitary bees - benefitting pollinator conservation.

Whilst many studies have focused on how floral resources may increase pollinator abundance (Chapter 4), it is generally unknown if observed increases in abundance are due to a transient movement of bees between patches of forage or due to an actual increase in colony development (Holzschuh *et al.*, 2016). Likewise, mass-flowering crops are frequently overlooked as a nutritional resource for pollinators despite being the intended forage resource for growers wishing to obtain pollination services. Therefore, by combining empirical data on courgette nectar and pollen with model simulations (using the novel bumblebee model *Bumble*-BEEHAVE), Chapter 5 quantified and simulated for the first time, the importance of courgette as a mass-flowering forage resource for bumblebees.

Whilst *B. terrestris* showed a strong fidelity to courgette flowers' bountiful nectar, no pollen loads from returning foragers were courgette pollen. Despite courgette being relatively high in protein (Petersen, Reiners and Nault, 2013), it's large sticky grains may make it difficult for the bees to collect (Vaissière and Vinson, 1994) and whilst *B. terrestris* has been observed to collect *Cucurbita* pollen in flight cages (Vaissière and Vinson, 1994), no studies have observed *B. terrestris* collecting cucurbit pollen in open fields. Therefore, *B. terrestris* may avoid collecting *Cucurbita* pollen, since as a generalist species it can visit alternative, more easily obtainable pollen (in open field settings). Nonetheless, early season courgette was shown to increase the number of hibernating queens, colonies, and adult workers, but not colony size or nectar or pollen

stores per bee. Courgette has the potential to improve bumblebee population dynamics however, bees can only benefit from this transient nectar source if alternative floral resources (particularly pollen) are also available to fulfil bees' nutritional requirements in space and time. Wild flowers could also attract bumblebees into courgette fields (Chapter 4) as they were shown to be the most important predictor of bumblebee abundance at courgette flowers. As bumblebees have been observed to stay constant to an area of good forage (Osborne *et al.*, 1999) it is likely that they were foraging between wild and crop flowers (due to their phenology) at a specific locality, thus supporting Chapter 5, which showed *B. terrestris* 'switching' to wild flowers. Therefore, courgette fields with additional wild flowers may simultaneously improve pollination services *and* bumblebee populations.

Can we improve yields of other pollinator-dependent crops?

Promoting growers' agricultural resilience by understanding crops' pollination requirements is essential for pollinator-dependent food security. Indeed, discovering that courgette was less dependent on pollination than previously thought (Chapter 3) led to a systematic review and meta-analysis on studies inducing parthenocarpy in crop species, which like courgette are also believed to be pollinator-dependent (Chapter 2). Indeed, whilst many ecological studies advocate increasing the 'supply' of pollinators (wild or managed) to improve crop yields (Chapters 3, 4, and 5), there has been little focus on altering a crop's 'demand' for pollinators.

The systematic review in Chapter 2 identified 161 studies on parthenocarpy taking place on 40% of crops for which an increase in the supply of pollinators is the only approach suggested by ecologists to improve crop yields. Even single

successes presented in the meta-analysis could lead to profound changes in production of certain crops, for example nearly all bananas on the global market are of the Cavendish variety, selectively bred to be parthenocarpic. Indeed, three species included in Chapter 2 occur in the top twenty crops for global production (Mt/yr in Klein *et al.* 2007) (tomato #12; watermelon #15; apple #19) and are therefore highly likely to have biotechnological routes explored to increase their yields.

The results of Chapter 2 support the conclusions of Klein *et al.* (2007) and Melathopoulos, Cutler & Tyedmers (2015), that to get a more complete picture, varietal information is required – both in terms of pollinator dependence, but also in terms of choices that farmers are making. Realistically the best way of obtaining this information is if the pollination requirements of each variety are tested by institutes conducting variety trials and that this information, alongside quantities sold, are made freely available.

Parthenocarpic crop species could ensure food security in the face of pollinator decline or changing pollinator distributions, since the need for pollination is removed. Therefore, producers could extend their growing seasons in otherwise adverse climatic and environmental conditions, furthering their economic advantage and agricultural resilience, which will ultimately improve food security. However, parthenocarpy should not be used as a panacea for agricultural success especially since biodiversity decline in agricultural landscapes is often an indicator of poor ecosystem health, which can also cause poor fruit set.

Indeed parthenocarpy may further the ‘pollinator crisis’ as the imperative for pollinator conservation is removed as our dependence on pollinators is reduced

(Brown *et al.*, 2016). This could affect pollination of non-parthenocarpic pollinator-dependent crops as well as wild plants. Whilst parthenocarpy may be contentious for some, environmental and technological solutions should be used in tandem to ensure that the best possible crop yields can be obtained in regions where they are needed most. Likewise, evidence of the effectiveness of different approaches to improve pollinator-dependent crop yields must be brought together for accurate valuations of ecosystem services and to ensure that decision-making is not skewed by one-sided arguments simply based on natural capital alone.

Recommendations

Because of this thesis four key management recommendations have been identified, which whilst aimed at growers, are also relevant to scientists and conservationists working in pollinator-dependent crop systems.

1) Parthenocarpy has the potential to lower a crop's demand for pollinators, whilst extending current geographic and climatic ranges of production. Thus, **growers may wish to use parthenocarpic varieties, in combination with other environmentally considerate practices**, to improve food security and their economic prospects.

2) Managed colonies of *B. terrestris* or *A. mellifera* could increase yields at sites with low levels of wild pollinators, although the evidence for this was not shown in this thesis. However, **growers may wish to consider more sustainable pollination management than adding managed bee colonies to fields** (see recommendation 3). As increasing the species richness and abundance of wild pollinators could buffer against potential fluctuations within individual pollinator populations.

3) **Species-rich wild flowers are an effective way of boosting the abundance of bumblebees which are important pollinators of courgette.** Therefore, allowing uncultivated areas around the crop to be colonised by wild flowers and maintaining/ protecting existing floral resources will ensure that courgette fields have high pollination services.

4) **Early season courgette crops may improve bumblebee populations.** This is because crop flowering coincides with when bumblebee foragers are most active, boosting the number of pollinators in the crop, thus increasing the potential for pollination *and* more food to be brought back to the colony which will improve bumblebee populations. However, as bumblebees don't collect courgette pollen and courgette nectar is only available for a short time period (with a day and season) other floral resources, such as those found on well-maintained hedgerows and on wild-flower strips must be available to fulfil bees' requirements beyond the crop. **Late flowering courgette may benefit from being planted in adjacent fields to early flowering courgette** to utilise increased numbers of foragers and further benefit pollinator populations. In doing so, growers may be able to simultaneously improve pollination and bumblebee populations in intensive farmland.

Future research

Aside from the knowledge gaps already identified, this thesis has resulted in two key avenues for future research:

1) The mechanism of pollination:

Whilst pollination clearly affects cucurbit yield, other environmental factors such as water, nutrients, pests, and disease will also affect the quantity and quality of yield (Figure 1.2~~Figure 1.2~~, Chapter 1) (Bos *et al.*, 2007). It would be useful to

gain a greater understanding of how a plant's physiological health interacts with the level of pollination it receives to influence yield. For example, Klein *et al.* (2014) found interacting effects of pollination, water, and nutrients on fruit set in almond. Indeed, sunflower yield was greatest with high levels of pollination and nitrogen (Tamburini, Lami and Marini, 2017), and oilseed rape yield was greatest with less pollinator-dependent varieties and high levels of nitrogen (Marini *et al.*, 2015). These findings suggest optimal pollination levels or reduced pollinator dependency may be able to compensate for lower levels of nitrogen, and conversely, that high levels of nitrogen may be able to compensate for lower levels of pollination or higher pollination dependency. Consequently, integrating environmental and agronomic management by combining pollination management with decisions about what nutrients to apply and varieties to grow could be an effective way of improving growers' agricultural resilience.

Drought stress, nutrient deficiencies, and diseases such as cucumber mosaic virus and powdery mildew are relatively common in courgette production (more so than direct pest damage) (Agriculture and Horticulture Developement Board, 2013). Since biological control may reduce aphid populations, which are common vectors of cucumber mosaic virus, and fungicides can reduce powdery mildew, **fully-factorial experiments could be established which test the effect of disease control (biological and/or chemical) in relation to different levels of pollination (i.e. hand, open, and no pollination), nutrient (e.g. fertiliser use), and water availability (e.g. irrigation and/or rain covers) on fruit set.**

2) The management for pollination:

It is rarely considered if these factors, which can be provided by regulating services, interact synergistically or antagonistically to affect fruit set (Bommarco, Kleijn and Potts, 2013). A more complete understanding of this is vital to ensure that ecologists don't promote the conservation of one ecosystem service at the expense of another and that growers are able to prioritise key services in their management for optimal crop yields. It would be interesting to understand how different growers, e.g. growing different species of pollinator-dependent crops, perceive pollination in relation to their crops' level of pollinator dependence, as well as how the different factors affecting fruit set are prioritised in farm management, relative to the empirical evidence. **A social survey could be conducted to explore how growers' attitudes towards pollinators correspond to their management actions and if growers identify any key barriers or opportunities to integrating pollination in their management for optimal crop yields.** To date farmer surveys have only focused on ecosystem services in isolation. For example, surveys identified that achieving consistent and reliable pollination is a priority for blueberry growers in Michigan and Florida in the US (Integrated Crop Pollination Project, 2016a and 2016b). However, there is no way of knowing how much of a priority pollination is to these blueberry growers, relative to all the other factors which may affect yield such as pest control or soil quality. This information is critical to understand the likelihood of growers adapting existing, or adopting new, sustainable pollination services.

Simulations in BEESTEWARD:

Provisioning pollinator habitat has been shown to improve pollinator visitation to crop flowers and therefore increase yield (Blaauw and Isaacs, 2014; Pywell *et al.*, 2015). Since BEE-STEWARD can simulate the effect that provisioning pollinator habitat could have on bumblebee population dynamics and their visitation to crop flowers, **simulations could be run to explore the optimum location and size of pollinator habitat.** Pollinator habitat could include allowing wild flowers to grow in cropped areas, maintaining hedgerows, planting wild flower strips etc. These findings would add to a growing body of literature exploring the effects of landscape context (Scheper, Holzschuh, Kuussaari, Potts, Rundlöf, Smith and Kleijn, 2013; Grab *et al.*, 2018; Herbertsson *et al.*, 2018) and intervention size (Holland *et al.*, 2015; Rundlöf, Lundin and Bommarco, 2018) on the overall effectiveness of pollinator management interventions.

However, it can be difficult to determine if these pollinator management practices improve pollinator populations and therefore benefit species conservation. **Simulations could be run to explore how the quantity and heterogeneity of pollinator habitat may affect bumblebee populations *and* bumblebee visitation rates to crop flowers.** These findings could support empirical data which suggest that whilst small areas of mass-flowering crops may 'concentrate' pollinator abundance from semi-natural habitats and therefore enhance pollination services, large areas of mass-flowering crops may 'dilute' pollinator abundance and therefore reduce pollination services. By comparing indicators of bumblebee population success such as the number of over-wintering queens, with forager numbers, it would be possible to determine if any concentration or dilution effects were due to a transient movement of

pollinators between patches of forage or due to an actual increase in pollinator populations.

Since the phenology and nutritional value of mass-flowering crops and wild flower species can affect bumblebee population dynamics and therefore pollination services (Vaudo *et al.*, 2015; Grab *et al.*, 2017). **BEE-STEWARD could simulate the effect that different mass-flowering crop species, e.g. oilseed rape and field beans, and/ or different types of semi-natural habitat, e.g. heathland versus woodland, may have on bumblebee population dynamics and pollination services.** This information would benefit conservationists and growers wishing to provide cost-effective sustainable pollination management at a landscape scale (Landis, 2017; Grab *et al.*, 2018).

Conclusions

This thesis highlights the importance of pollination for improving yields, even when over half of fruit set can be achieved via parthenocarpy. The high abundance and pollination efficiency of *B. terrestris* (alongside other pollinator species) in field grown courgette was enough to fulfil its pollination requirements. However, since the total economic value of insect pollination to courgette is estimated to be worth £3,398 per ha (Knapp and Osborne, 2017), growers may wish to preserve their wild flowers within, and on the edge of fields as a way of attracting pollinators into courgette fields. In doing so growers will also support pollinators' nutritional requirements beyond those already provided by courgette. Indeed, courgette fields studied in this thesis, with abundant and diverse wild flowers can simultaneously improve pollination services to courgette *and* bumblebee populations in the surrounding landscape which will benefit courgette production in subsequent years. Nonetheless, for food security to be maximised, parthenocarpic varieties should be used in combination with these pollinator supportive practices (Figure 6.1) to ensure that the best possible yields can be obtained.

7. Appendices

Plate 12 *Eristalis arbustorum* L. collecting nectar from hogweed on a courgette field margin. Photograph by Daphne Wong.

Appendix 1. Thesis introduction

1.1 Pollination Biology of Courgette, Cucurbita pepo

Pollinators vary in the way that they travel to collect nectar in staminate and pistillate flowers because the nectar is in different places (Figure A 1.1). For example, in staminate flowers, bees are forced into a vertical position to gather nectar which means that pollen adheres to the bee's backs. Once out of the staminate flower, bees tend to sit on a flower or leaf and clean excess pollen grains from themselves using their back legs (see also Figure A 4.5). This usually happens in the first hour of anthesis when pollen grains are plentiful. Nonetheless, many pollen grains will remain on the bees. Once inside a pistillate flower, bees unload their pollen grains on to the stigma as they make their way to the base of the corolla. Here, two to three bees may collect nectar at the same time and continue to release more pollen grains as they move symmetrically around the whole circumference of the corolla (Figure A 1.1).

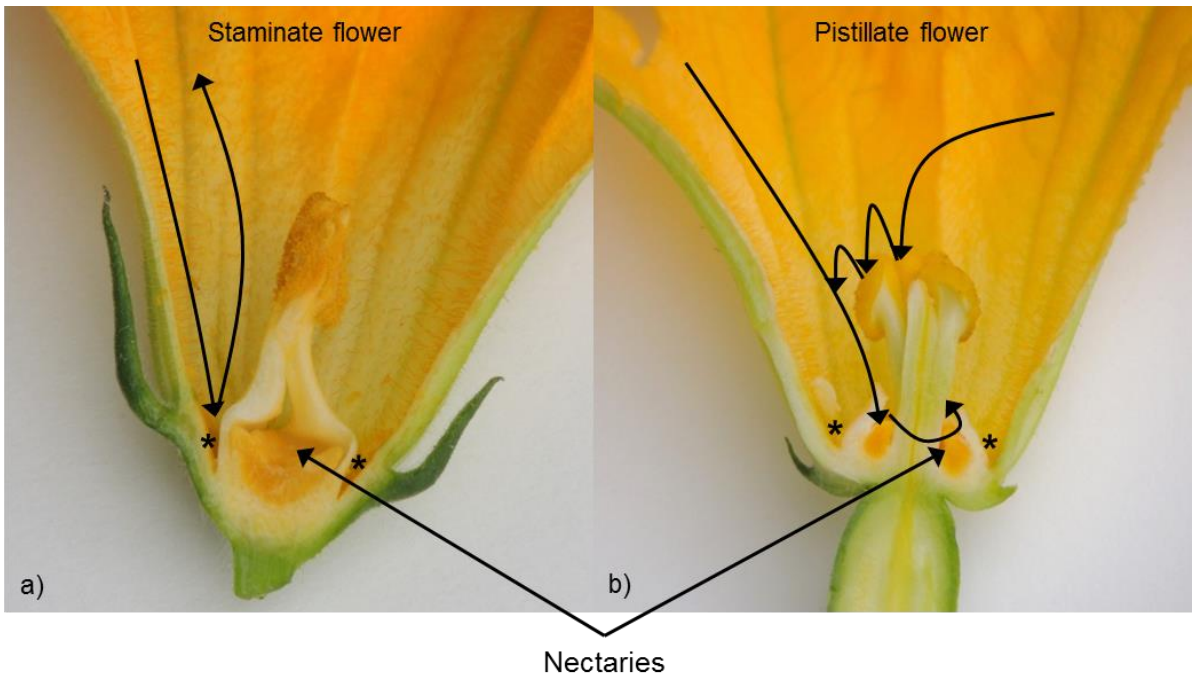

Figure A 1.1 Morphology of staminate (a) and pistillate (b) flowers. The nectaries are shown and arrows show the path of bees collecting nectar. Asterisks (*) show where pollen accumulates. Diagram modified from (Nepi, Massimo and Pacini, 1993).

Appendix 2. Re-evaluating strategies for pollinator-dependent crops: how useful is parthenocarpy?

2.1 Studies identified by the key word search which investigate methods to induce parthenocarpy.

Crop species with no references demonstrate current areas where parthenocarpy has not been used commercially or experimentally. References in bold are included in the meta-analysis, references in black were identified in the systematic review but not included in the meta-analysis (i.e. they did not fulfil all the search requirements), and references in blue only had an abstract available.

Species were pollinator-dependent if animal pollination was shown to increase production (fruit set, fruit weight and/or quality, seed number and/or seed quality, and/or increased pollen deposition (an indirect measure)) for at least one variety per crop (Klein *et al.*, 2007). Only crop species with essential, great, modest, and/or little dependence on pollinators were included in analyses. Essential = pollinators essential for most varieties (production reduction by ≥ 90% comparing experiments with and without animal pollinators. Great = great production increase/ animal pollinators are strongly needed (40 - <90% reduction). Modest = modest production increase/ animal pollinators are clearly beneficial (10 - < 40% reduction) (Klein *et al.*, 2007).

Seed and nut crops are absent from this table as parthenocarpy (therefore, seedlessness) would not be a desired trait. Entries are alphabetically ordered by genus.

Table A 2.1 Pollinator-dependent crops, as defined by Klein *et al.* (2007) and studies (identified by the key word search) which investigate methods to induce parthenocarpy.

		From Klein et al 2007		Studies which promote the effect of parthenocarpy on yield identified found from the systematic review of the literature. Studies in bold were included in the meta-analysis, studies in black were included in the systematic review and studies in blue only had abstracts available.		
Crop species	Crop name	Requirement for animal pollination	Rank# in list of top global crops Mt/yr	Selective breeding	Genetic engineering	Growth hormones
Abelmoschus esculentus	Okra	modest	#56			
Actinidia deliciosa	Kiwifruit	essential				**Iwahori *et al.* 1988** Ohara *et al.* 1997
Annona squamosa	Atemoya, Cherimoya, Custard apple	essential			Lora *et al.* 2011	
Asimina triloba	Pawpaw, Indiana banana	essential				
Averrhoa carambola	Carambola, Starfruit	great				

Capsicum annum, C. frutescens	Chilli pepper, Red pepper, Bell pepper, Green pepper, Allspice, Pimento	little	#33	Carrizo 2011 Honda *et al.* 2012 **Tiwari *et al.* 2007**		Balakbir *et al.* 1998 Gustafson 1936 Heuvelink & Korner 2001 Thanopoulos *et al.* 2013 **Tiwari *et al.* 2012** **Wien & Zhang 1991**
Carica papaya	Papaya	little	**#52**	**Rimberia *et al.* 2007**		
Citrullus lanatus	Watermelon	essential	#15			**Hayata *et al.* 1995** Huitrón *et al.* 2007 Kwon *et al.* 2006 **Maroto *et al.* 2005** Sedgley *et al.* 1977 Hayata *et al.* 1994 Hikosaka *et al.* 2015 Newbury *et al.* 1977 Pak 1993 Miguel *et al.*2000
Citrus spp.	Bergamont, Chinotto, Citron, Clementine, Grapefruit, Kumquat, Lemmon, Lime, Manderine, Orange,	little parthenocarpy listed as a breeding mechanism	#13	Mesejo *et al.* 2013		García-Martínez & García-Papí 1979 Guardiola *et al.* 1993 Talon *et al.* 1992

	Pomelo, Tangerine					
Cucumis melo	Cantaloupe, Melon	essential	#29			**Hayata *et al.* 2000** Risser 1976 Masuda 1990
Cucumis sativus	Cucumber, Gherkin	great	#24	Kushnereva 2008 Li *et al.* 2014 Shaw *et al.* 2007 Sun *et al.* 2006 Yan *et al.* 2012 Dean *et al.* 1983 Dennijs *et al.* 1991	Yin *et al.* 2006	Fu *et al.* 2008 Hikosaka & Sugiyama 2015 Kim *et al.* 1992 Shin *et al.* 2007
Cucurbita maxima, C. mixta, C. moschata, C. pepo	Pumpkin, Squash, Gourd, Marrow, Courgette	essential	#36	Kurtar 2003 Martínez *et al.* 2014 Nogueira *et al.* 2011 Robinson & Reiners 1999		Martínez *et al.* 2013 Yu 1999
Durio zibethinus	Durian	great				
Eriobotrya japonica	Loquat, Japanese plum, Japanese mediar	great			Tao *et al.* 2015	**Mesejo *et al.* 2010** Ding 1988
Fagopyrum esculentum	Buckwheat	great				

Feijoa sellowania	Feijoa	great				
Ficus carica	Fig	modest				Blondeau and Crane 1949 Crane 1964 Crane *et al.* 1948
Fragaria spp.	Strawberry	modest			**Mezzetti *et al.* 2004**	Mudge *et al.* 1981
Lagenaria siceraria	Bottle gourd	Not in Klein *et al.* 2007				**Yu 1999**
Luffa acutangula	Luffa	Not in Klein *et al.* 2007				**Bisaria 1977**
Malus domestica	Apple	great parthenocarpy listed as a breeding mechanism	#19	**Watanabe *et al.* 2008**	Yao *et al.* 2001	**Watanabe *et al.* 2008** Bangerth 1994 Bangerth *et al.* 1994 Bukovac 1963 Goldwin et al Goldwin et al 1989 Greene 1980 Williams 1980 Luckwill 1960
Mangifera indica	Mango	great	#30		**Ogata *et al.* 2010**	Ogata 2009 Perez-Barraza *et al.* 2015
Manilkara zapota	Sapodilla	essential				

Passiflora edulis	Passionfruit	essential				
Persea americana	Avocado	great				
Prunus armeniaca	Apricot	great				Crane *et al.* 1960
Prunus avium	Sweet cherry	great				Crane *et al.* 1960
Prunus cerasus	Sour cherry	great				Crane *et al.* 1960
Prunus domestica, P. spinosa	Plum, Greengage, Mirabelle, Sloe	great	#48			Crane *et al.* 1960 Hartmann 1984 Jackson 1968
Prunus persica	Peach, Nectarine	great	#42			Crane *et al.* 1960
Psidium guajava	Guava, Guayaba	modest				
Punica granatum	Pomegranate	modest				
Pyrus communis	Pear	great	#37	Nishitani *et al.* 2012		**Lafer 2008** **Niu *et al.* 2015** Yarushnykov & Blanke 2005 Zhang *et al.* 2008 Luckwill 1960 Yamada *et al.* 1991

Ribes nigrum, R. rubrum,	Black currant, Red currant	modest				
Rosa spp.	Rose hips, Dogroses	great				
Rubus ideaus, R. fruiticosus, R. chamaemorus , R. flagellaris, R. trivalis	Raspberry, Blackberry, Cloudberry, Northern Drewberry, Southern Drewberry	great			**Mezzetti *et al.* 2004**	**Junttila *et al.* 2002**
Solanum lycopersicum *parthenocarpy listed as one of breeding mechanisms	Tomato	little	#12	**Baggett *et al.* 1997** Charbeonboonsit *et al.* 1985 Costa *et al.* 1992 Dutta *et al.* 2013 **Fos *et al.* 2003** Gorguet *et al.* 2005 **Habashy *et al.* 2004** Mazzucato *et al.* 1998 Mohamed 1998 Philouze *et al.* 1986 Sugahara *et al.* 2002 Tang *et al.* 2015	**Barg & Salts 2000** Carmi *et al.* 2003 Carrera *et al.* 2012 **Ficcadenti *et al.* 1999** **García-Hurtado *et al.* 2012** Goetz *et al.* 2007 Ingrosso *et al.* 2011 Marti *et al.* 2007 **Medina *et al.* 2013** **Molesini *et al.* 2009** Pandolfini *et al.* 2002 **Rotino *et al.* 2005** Schijlen *et al.* 2007 **Shabtai *et al.* 2007**	Fos *et al.* 2003 **Gemici *et al.* 2006** **Goetz *et al.* 2007** Gustafson 1936 **Karapanos *et al.* 2013** **Nandwani *et al.* 2014** **Ramin 2003** Rounis *et al.* 2015 **Serrani *et al.* 2008** Aguero *et al.* 2007 Alabadi *et al.* 1996 Alabadi *et al.* 1998 Ampomah-Dwamena *et al.* 2002

						Bunger-Kibler *et al.* 1982 Costa et al 1985 El-Habbasha *et al.*1999 Gorecka *et al.* 1987 Gustafson 1960 Mapelli *et al.* 1987 Mariotti *et al.* 2011 Matsuo et al, 2012 Mazzucato *et al.* 1999 Mignolli *et al.* 2012 Shinozaki *et al.* 2015 Sjut 1982 Sjut 1984 varga et al 1986 Gelmesa *et al.* 2013
Solanum melongena	Aubergine	modest	#27	Kikuchi *et al.* 2008 Mori *et al.* 2013 **Saito *et al.* 2009** Boyaci 2009 Takeshi *et al.* 2010	**Acciarri *et al.* 2002** **Donzella *et al.* 2000** **Rotino *et al.* 1997**	Boyaci *et al.* 2011 Gustafson 1936 Sarma *et al.* 1997 Sidhu 2007
Solanum muricatum	Pepino dulce, Sweet cucumber	Not in Klein *et al.* 2007		Nuez *et al.* 1998 Prohens *et al.* 2002 Prophens and Nuez 2000		**Ercan & Akilli 1996** Maroto *et al.* 1997

				Rodriguez-Burruezo et al 2011		
Solanum quitoense	Naranjillo	great				
Sorbus aucuparia	Rowanberry	essential				
Vaccinium corymbosum, V. angustifolium, V. ashei, V. myrtillus	Highbrush blueberry, Lowbrush blueberry, Rabbiteye blueberry, Bilberry	great				Junttila *et al.* 2002
Vaccinium macrocarpon, V. oxycoccus	American cranberry, European cranberry	great				Devlin & Demoranville 1967

2.2 Final dataset used in the meta-analysis, alphabetically ordered by genus.

Table A 2.2 Final dataset used in the meta-analysis, alphabetically ordered by genus.

Species	Method to induce Parthenocarpy	Test environment	Measure	Effect Size	Variance
Actinidia chinensis	HA	NP conditions	Sugars	-0.082	0.021109
Actinidia chinensis	HA	NP conditions	Yield	1.996201	0.245295
Capsicum annuum	SB	OP conditions	Yield	1.836082	0.028712
Capsicum annuum	HA	NP conditions	Yield	1.33715	0.005758
Capsicum annuum	HA	HP conditions	Yield	-0.34255	0.012835
Carica papaya	SB	OP conditions	Yield	1.136643	0.003864
Citrullus lanatus	HA	HP conditions	Sugars	-0.20544	0.006123
Citrullus lanatus	HA	HP conditions	Yield	0.155029	0.006113
Citrullus lanatus	HA	OP conditions	Sugars	0.051341	0.021092
Citrullus lanatus	HA	OP conditions	Yield	-0.92558	0.007337
Cucumis melo	HA	NP conditions	Sugars	13.91252	0.159346
Cucumis melo	HA	NP conditions	Yield	10.83453	0.099127
Cucumis melo	HA	OP conditions	Sugars	0.460481	0.006492
Cucumis melo	HA	OP conditions	Yield	0.275551	0.006385
Eriobotrya japonica	HA	OP conditions	Sugars	0.086106	0.001266
Eriobotrya japonica	HA	OP conditions	Yield	-2.20575	0.002034
Fragaria ananassa	GM	OP conditions	Sugars	0.057971	0.002531
Fragaria ananassa	GM	OP conditions	Yield	0.91286	0.001397
Fragaria vesca	GM	OP conditions	Sugars	0.164231	0.006346
Fragaria vesca	GM	OP conditions	Yield	2.621295	0.003919
Lagenaria siceraria	HA	NP conditions	Yield	2.876362	0.003216
Luffa acutangula	HA	OP conditions	Yield	1.4368	0.007957
Malus pumila	HA	NP conditions	Yield	4.924251	0.012747
Malus pumila	SB	NP conditions	Yield	7.911708	3.269794
Mangifera indica	HA	OP conditions	Sugars	0.81371	0.006848
Mangifera indica	HA	OP conditions	Yield	1.687746	0.008576
Pyrus communis	HA	OP conditions	Yield	0.582146	0.001236
Pyrus communis	HA	OP conditions	Yield	0.393657	0.001201
Rubus chamaemorus	HA	HP conditions	Yield	0.121166	0.004547
Rubus idaeus	GM	OP conditions	Sugars	2.470727	0.005575
Rubus idaeus	GM	OP conditions	Yield	0.798029	0.001707

Solanum lycopersicum	SB	OP conditions	Yield	5.113391	0.003374
Solanum lycopersicum	GM	OP conditions	Sugars	0.440201	0.006922
Solanum lycopersicum	GM	OP conditions	Yield	0.605605	0.007052
Solanum lycopersicum	GM	HP conditions	Sugars	1.283145	0.005336
Solanum lycopersicum	GM	HP conditions	Yield	1.650157	0.006492
Solanum lycopersicum	GM	NP conditions	Sugars	15.43655	131.9856
Solanum lycopersicum	GM	NP conditions	Yield	10.62201	12.64204
Solanum lycopersicum	HA	NP conditions	Yield	3.61653	0.015346
Solanum lycopersicum	SB	NP conditions	Yield	3.204817	0.013882
Solanum lycopersicum	GM	OP conditions	Sugars	1.425102	0.01586
Solanum lycopersicum	GM	OP conditions	Yield	1.991905	0.009461
Solanum lycopersicum	GM	OP conditions	Sugars	3.43146	0.031267
Solanum lycopersicum	GM	OP conditions	Yield	1.070159	0.00723
Solanum lycopersicum	HA	OP conditions	Yield	1.336772	0.007737
Solanum lycopersicum	GM	NP conditions	Yield	2.094612	0.001646
Solanum lycopersicum	SB	OP conditions	Yield	0.484457	0.004744
Solanum lycopersicum	HA	OP conditions	Yield	3.244583	0.185242
Solanum lycopersicum	GM	OP conditions	Sugars	11.85771	0.234966
Solanum lycopersicum	GM	OP conditions	Yield	8.835926	0.136094
Solanum lycopersicum	GM	HP conditions	Yield	-3.62953	0.014606
Solanum lycopersicum	GM	NP conditions	Yield	36.08369	1.093537
Solanum lycopersicum	HA	OP conditions	Yield	1.186435	0.038214
Solanum lycopersicum	GM	NP conditions	Yield	2.020992	0.009014
Solanum lycopersicum	HA	OP conditions	Yield	4.556189	0.631613
Solanum lycopersicum	GM	OP conditions	Sugars	0.210216	0.000111
Solanum lycopersicum	GM	OP conditions	Yield	0.143855	0.00317
Solanum lycopersicum	HA	NP conditions	Yield	28.83752	0.829705
Solanum lycopersicum	GM	NP conditions	Sugars	-1.42147	0.001866
Solanum lycopersicum	GM	NP conditions	Yield	1.739234	0.006145
Solanum melongena	GM	OP conditions	Yield	1.444628	0.070885
Solanum melongena	SB	OP conditions	Yield	1.761513	0.01627
Solanum melongena	GM	NP conditions	Yield	3.679541	1.106564
Solanum melongena	HA	NP conditions	Yield	2.399622	0.121138
Solanum melongena	SB	NP conditions	Yield	-1.19352	0.016926
Solanum melongena	GM	NP conditions	Yield	4.184441	1.306233
Solanum melongena	SB	OP conditions	Yield	2.084884	0.007034
Solanum muricatum	HA	OP conditions	Yield	1.662862	0.037947
Solanum muricatum	HA	OP conditions	Yield	0.237487	0.004253

2.3 Forest plots showing effect sizes only from studies with complete data, i.e. without bootstrapping for missing standard deviations

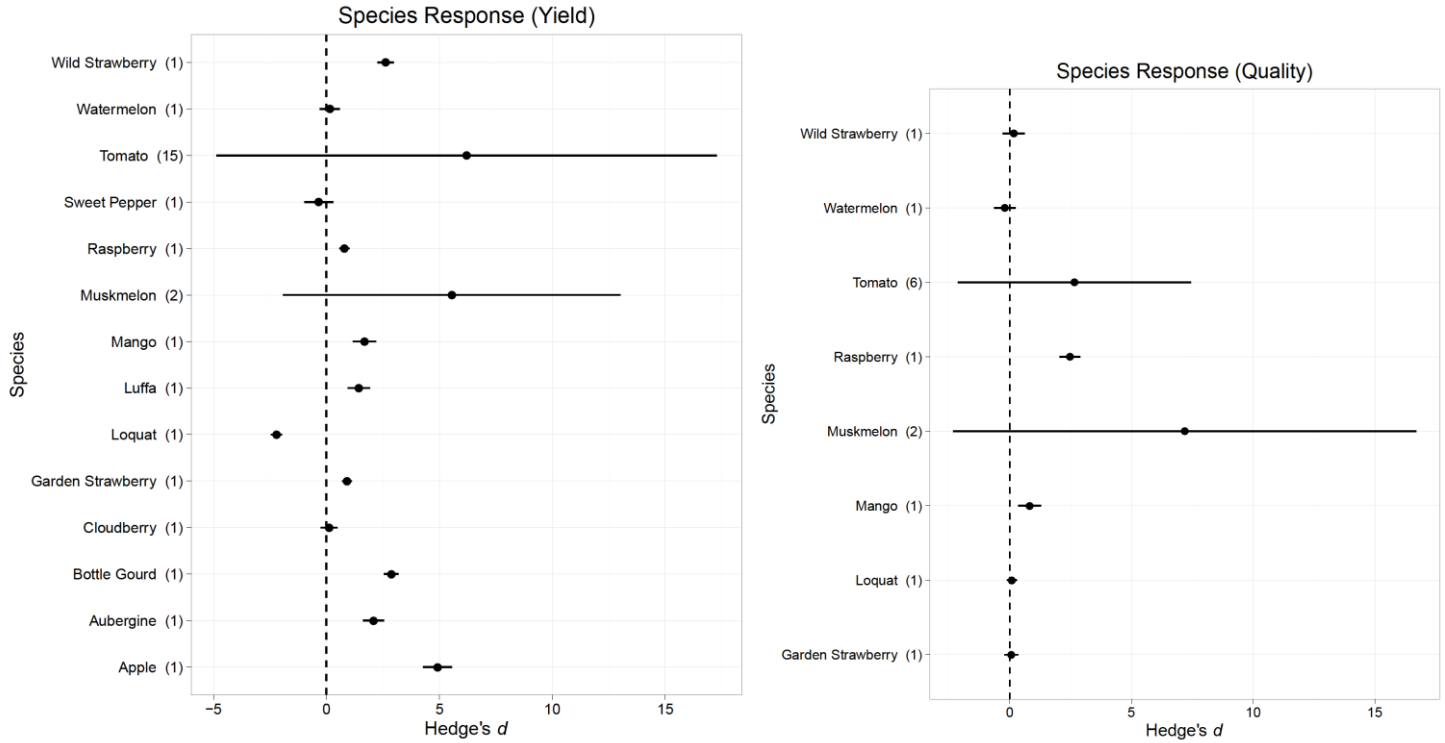

Figure A 2.1 Mean effect sizes for all methods combined to induce parthenocarpy (genetic modification, hormone application, and selective breeding) split by crop species (y axis) for (a) fruit quantity (b) fruit quality. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

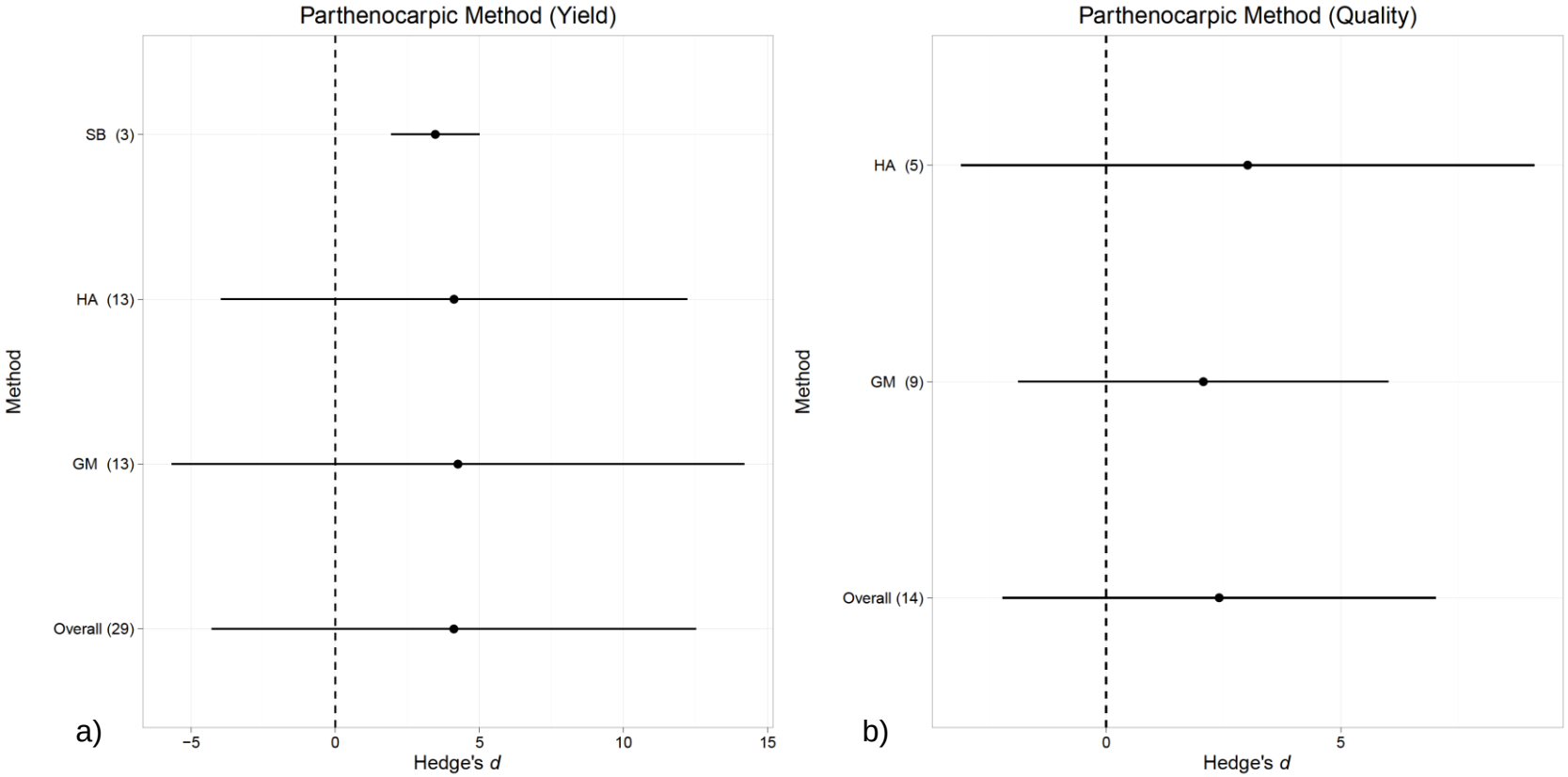

Figure A 2.2 Overall mean effect sizes and effect sizes of methods to induce parthenocarpy (genetic modification (GM), hormone application (HA), selective breeding (SB)) (y axis) for (a) fruit quantity and (b) quality for all crop species. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

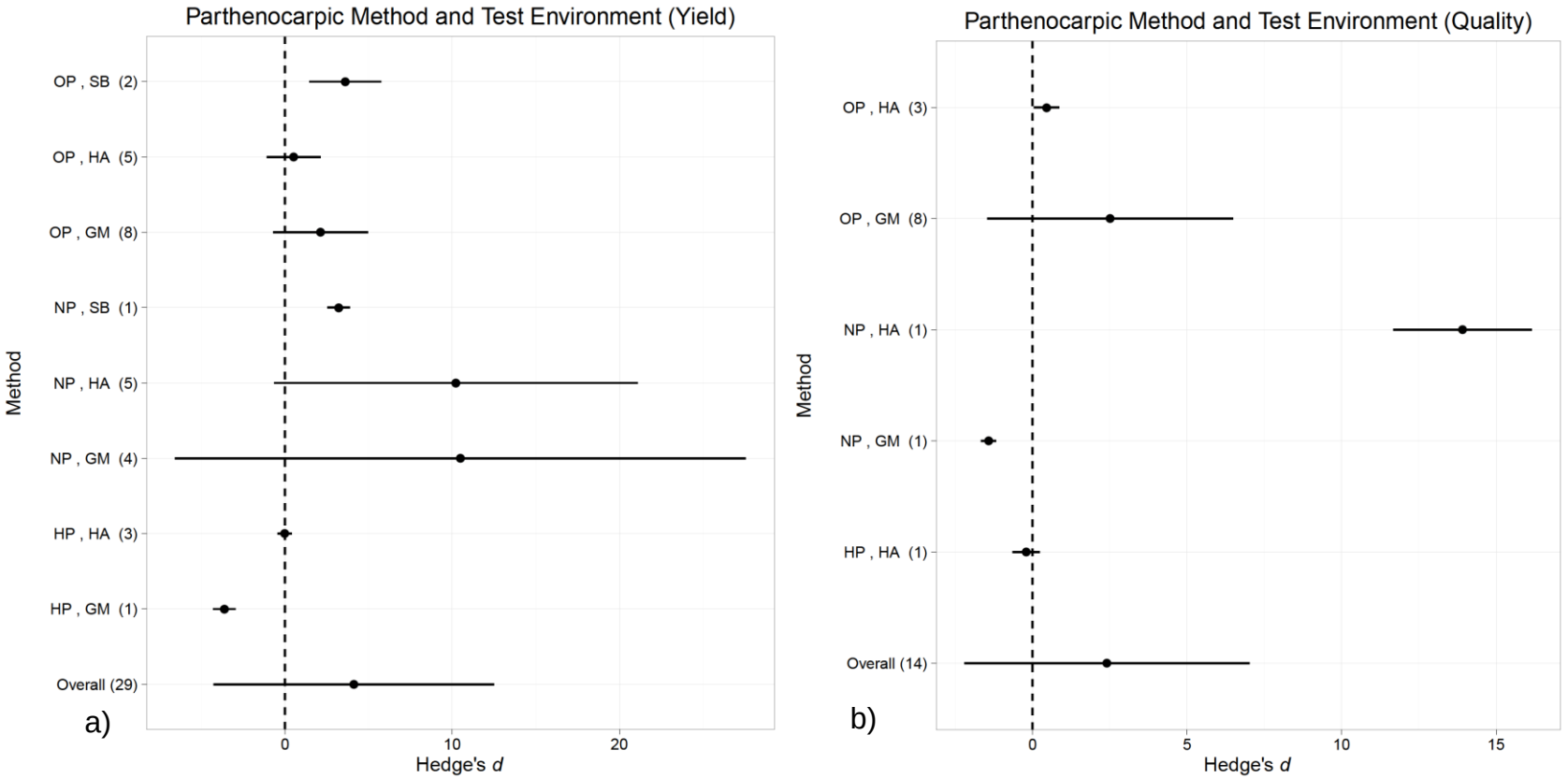

Figure A 2.3 Overall mean effect sizes and effect sizes of methods to induce parthenocarpy (genetic modification (GM), hormone application (HA), selective breeding (SB)) and test environment (NP, OP, and HP) (y axis) for (a) fruit quantity (b) fruit quality for all crop species. Error bars represent standard deviations. Sample size (number of effect sizes) are given in parentheses.

Appendix 3. Pollinator visitation to mass-flowering courgette and co-flowering wild flowers: implications for pollination and pollinator conservation

3.1 Nutrients and yield

Methods

Questionnaires were conducted to ensure farming intensity i.e. the type of farm machinery used during picking, the number of herbicide, fungicide and fertiliser applications and cropping practices such as planting dates, start, and end dates of picking were similar between sites. Sites differed slightly in the number of herbicide, fungicide and fertiliser applications and the period of time that courgettes were harvested.

As nitrogen, pH, phosphorous and potassium are important indicators of plant growth, a single homogenised soil sample was collected from four locations (halfway along pollinator transects) within the crop (2 - 5 cm deep). This was done three times within the blooming period, for each field, totalling 27 soil samples. All soil analyses were conducted using a professional agriculture field test kit (Hanna Instruments, Rhode Island, US) which used colourmetric tests for nitrogen, pH, and phosphorous and a turbidimetric test for potassium.

At the end of the season, each farmer provided data on their total marketable yield (kg) per field. This was then calculated as yield/Ha/day based on the area of the field and the number of days which it was cropped. Due to one farmer not providing data the final sample size of this stage of the analysis was 8.

Results

On average yield was greatest, but most varied in Cornwall, then Cambridgeshire, then Gloucestershire (Figure A 3.1). Across all sites, the average values for nutrients were: nitrogen 3.2 ± 0.3 (*SE*), phosphorous 2.5 ± 0.3, and potassium 2.5 ± 0.2.

Yield was best predicted by an increase in *Bombus* abundance per field margin (R^2 = 0.29) (Table A 3.1). The next best fitting model (an increase in crop solitary bee abundance) explained less of the overall variance (R^2 = 0.23) (Table A 3.1).

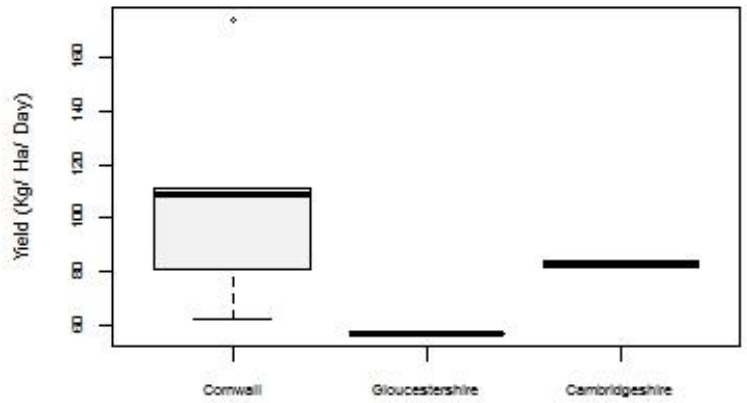

Figure A 3.1 Average marketable yield (Kg) per day for each region.

Table A 3.1 Top three best fitting models (AICc Δ< 2) describing the impact of field size, region, nutrients, pollinators (on field margins and within the crop) and floral resources (at a local, and landscape scale) for 3) courgette yield. Global model: Field size + Region + (Combined nutrients + Nitrogen + Potassium + Phosphorous) + Crop Floral Abundance + (*Apis mellifera* per field + *Bombus* on field margins per field + solitary bees on field margins per field + *Apis* in the crop + *Bombus* in the crop + solitary bees in the crop). Brackets indicate where due to multicollinearity, only one predictor variable for pollinators was entered into the model. Model results only show coefficient estimates for continuous fixed effects which occur in the best fitting models.

Intercept	Total *Bombus* in field margins	Total solitary bees in the crop	Nitrogen	df	AICc	ΔAICc	Weight	R^2
74.19	51.63			3	88.44	0.00	0.16	0.29
78.16		50.58		3	89.05	0.62	0.12	0.23
60.85			49.97	3	89.63	1.20	0.09	0.18

Pollinator abundance and species richness within the crop did not affect yield. Nonetheless, pollinator abundance on the edge of the field appeared to have a positive effect on yield in the most predictive model, but this was not statistically significant. *Bombus* abundance on the edge of the field was also more important than overall pollinator abundance/ species richness (across the whole field) and *Bombus* and *Apis* abundance/ species richness. However, this relationship was much weaker than has been observed in pumpkins (Petersen and Nault 2014) and berry crops (Blaauw and Isaacs 2014). These results support findings from oilseed rape which have shown a correlation between pollinator visitation and yield, but no evidence of yield declining with distance from the crop edge (Woodcock *et al.* 2016). Edge pollinator abundance was

also more important than species richness for improving yield, supporting previous findings in courgette (Knapp and Osborne 2017, Chapter 3). The yield models also showed that region, field size and nutrients were not important predictors of courgette yield. However, the limited sample size of eight fields meant that it was not possible to test the interaction of these predictors with pollinator abundance/ species richness, although it is known that nutrient availability can interact with pollination to influence yield (Klein *et al.* 2014, Marini *et al.* 2015). There was also no evidence of competition for soil resources and pollinators between wild flowers and courgette flowers, with neither floral abundance nor floral species richness negatively effecting courgette yield. This supports work in other species which have shown no negative effect of non-crop flowers on crop yield (Cierjacks *et al.* 2016). In this analysis, the weaker signal for yield is likely due to the way the analysis was structured; having single values for yield, per field, over the whole season, rather than measures of yield which directly followed the survey rounds at each site.

3.2 Supplementary figures

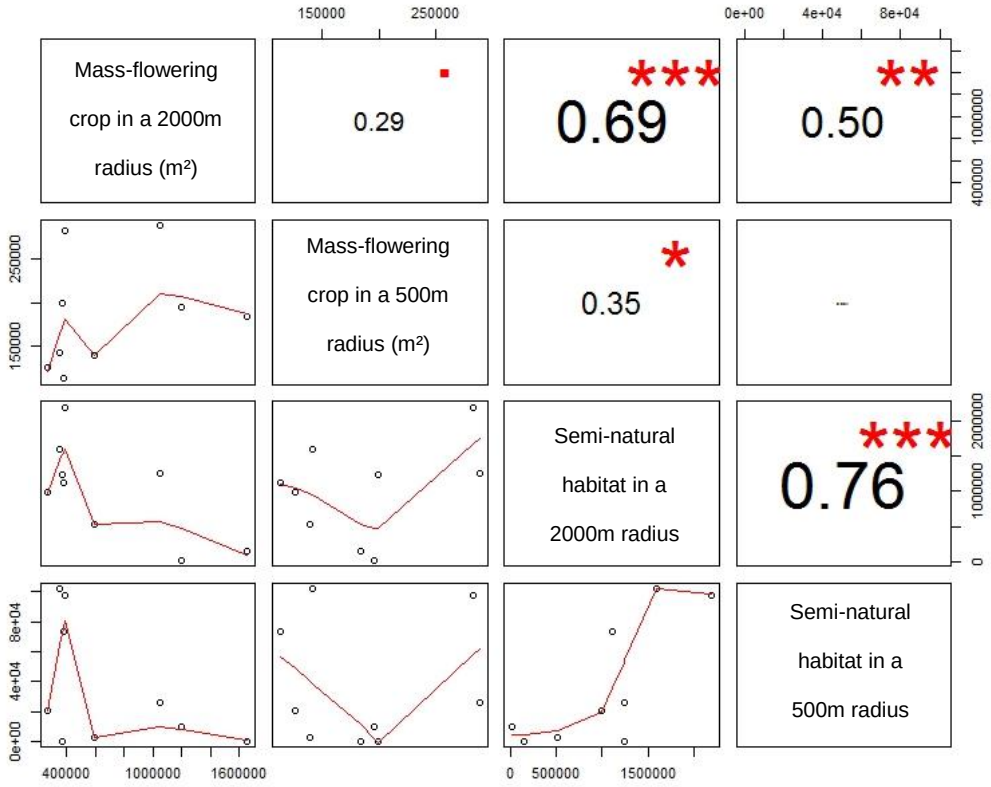

Figure A 3.2 Correlation matrix of explanatory variables. Abundance and species richness of floral resources, and semi-natural habitat and mass-flowering crops were always analysed in separate models due to collinearity.

3.3 Species list of bees and hoverflies recorded in courgette fields

Table A 3.2 Bees and hoverflies collected from pan traps in the cropped area and margins of courgette fields in 2016. Full methodology is described in Chapter 4. No species are of current conservation concern.

Species	Abundance
Honeybee	
Apis mellifera	10
Bumblebees	
Bombus hortorum	7
Bombus hypnorum	1
Bombus lapidarius	4
Bombus lucorum	2
Bombus pascuorum	2
Bombus pratorum	1
Bombus rupestris	2
Bombus terrestris	9
Bombus terrestris	1
Solitary bees	
Andrena angustior	6
Andrena bicolor	10
Andrena dorsata	1
Andrena flavipes	1
Andrena haemorrhoa	2
Andrena humilis	1
Andrena minutula	4
Andrena nigroaenea	1
Andrena subopaca	1
Halictus rubicundus	3
Hyleaus cornutus	1
Lasioglossum calceatum	13
Lasioglossum leucopus	4
Lasioglossum malachurum	20
Lasioglossum morio	2
Lasioglossum pauxillum	1
Lasioglossum punctatissimum	1
Lasioglossum smeathmanellum	13
Lasioglossum viollosulum	5
Megachile versicolor	1
Panurgus banksianus	4
Sphecodes pellucidus	2
Sphecodes puncticeps	1

Species	Abundance
Hoverflies	
Anasimyia contracta	1
Baccha obscuripennis	1
Cheilosia pagana	1
Episyrphus balteatus	8
Eristalis arbustorum	18
Eristalis interuptus	1
Eristalis pertinax	1
Eristalis tenax	7
Eupodes corollae	41
Helophilus pendulus	3
Melanstoma mellinum	4
Melanstoma scalare	1
Merodon equestris	8
Neoascia podagrica	3
Platycheirus albimanus	1
Platycheirus clypeatus	4
Platycheirus granditarsus	1
Sphaerophoria scripta	3
Syritta pipiens	4
Syrphus ribesii	2
Volucella bombylans	1

Table A 3.3.2 Bees recorded on transects in the cropped area and margins of courgette fields in 2016. Full methodology is described in Chapter 4. No species are of current conservation concern.

Species	Abundance
Honey bee	
Apis mellifera	369
Bumblebees	
Bombus hortorum	17
Bombus hypnorum	3
Bombus lapidarius	51
Bombus pascuorum	20
Bombus pratorum	2
Bombus terrestris/ lucorum	186

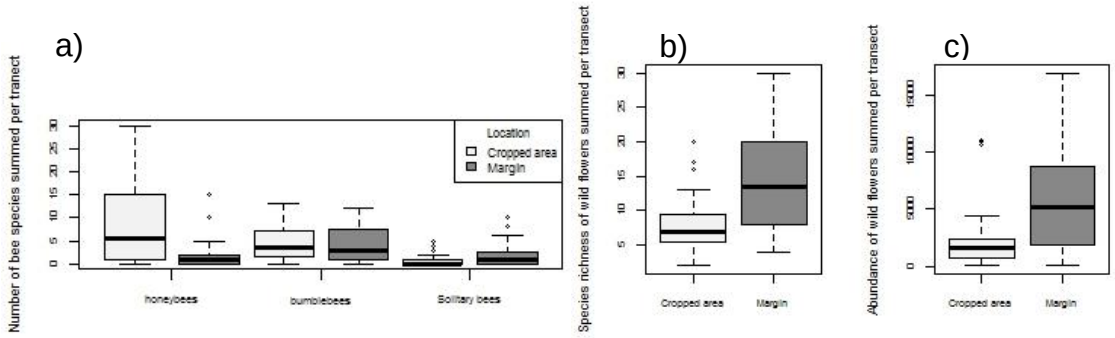

Figure A 3.3 Boxplots of a) honeybee, bumblebee, and solitary bee abundance, b) species richness of wild flowers, and c) abundance of wild flowers (boxplots represent median, interquartile range, and maximum and minimum recorded values). Figure 3.3a shows summed values from pan traps and transects and 3.3b - 3.3c summed values from transects, all from the three survey dates per season, divided into transects carried out within the cropped area (light grey) and on the field margin (dark grey).

Appendix 4. *Bombus terrestris* in a mass-flowering pollinator-dependent crop: A mutualistic relationship?

Table A 4.1 Input parameters for additional crop and habitat types for BEE-STEWARD.

Crop type	Flower species	Flower density (flowers/m²)	Pollen (g/ flower)	Nectar (ml/ flower)	Proportion of protein in pollen	Sugar concentration of nectar (mol/l)	Flowering start day	Flowering stop day	Corolla depth (mm)	Nectar flower volume (myl/ flower)	Internal flower handling time (seconds)	Notes
Courgette	Early courgette	3	0*	0.0765	0*	1.4660	163	212	0	76.47	2.5	1)
	Late courgette	3	0*	0.0765	0*	1.4660	213	251	0	76.47	2.5	
Improved grassland	Dandelion	0.05	0.0004	0.0005	0.0917	1.2947	1	364	1.2	0.4702	0.6	2)
	White clover	2.34	0.0004	0.0007	0.2307	0.9803	151	272	2	0.6666	0.6	
Heath	Ling	465	0**	0.0001	0**	1.17	182	273	4	0.0787	0.6	3)
	Bell Heather	7.17	0**	0.0002	0**	1.17	121	334	5.5	0.1997	0.6	
	Cross-leaved Heather	2.14	0**	0.0002	0**	1.17	152	273	6.5	0.1742	0.6	
	Bilberry	9.23	0**	0.0026	0**	1.17	91	181	5	2.6215	0.6	

Notes (Table A 4.1):

1) Empirical observations. * Pollen values set to 0 as no bees were observed bringing back courgette pollen to their colonies.

2) Nectar and pollen were already in the model; however, flower species and flower density were based on empirical observations.

3) Data from Baude et al. (2016). ** No pollen data available for these species.

Table A 4.2 Plant species identified from a subsample of pollen loads n=56, from the total 394 loads collected. None of the yellow pollen was courgette pollen.

Species name	Common name	Number of pollen loads
Brassica spp.	Brassica spp.	15
Rubus fruticosus	Bramble	11
Papaver rhoeas	Common poppy	7
Veronica filiformis	Speedwell	4
Helianthemum chamaecistus	Common rockrose	3
Linaria vulgaris	Common toadflax	3
Verbascum thapsus	Great mullein	3
Echium vulgare	Viper's bugloss	2
Hedera helix	Common ivy	2
Ribes sanguineum	Flowering currant	2
Calystegia sepium	Hedge bindweed	1
Centaurea cyanus	Cornflower	1
Centranthus ruber	Red valerian	1
Heracleum sphondylium	Hogweed	1

4.2 Supplementary figures

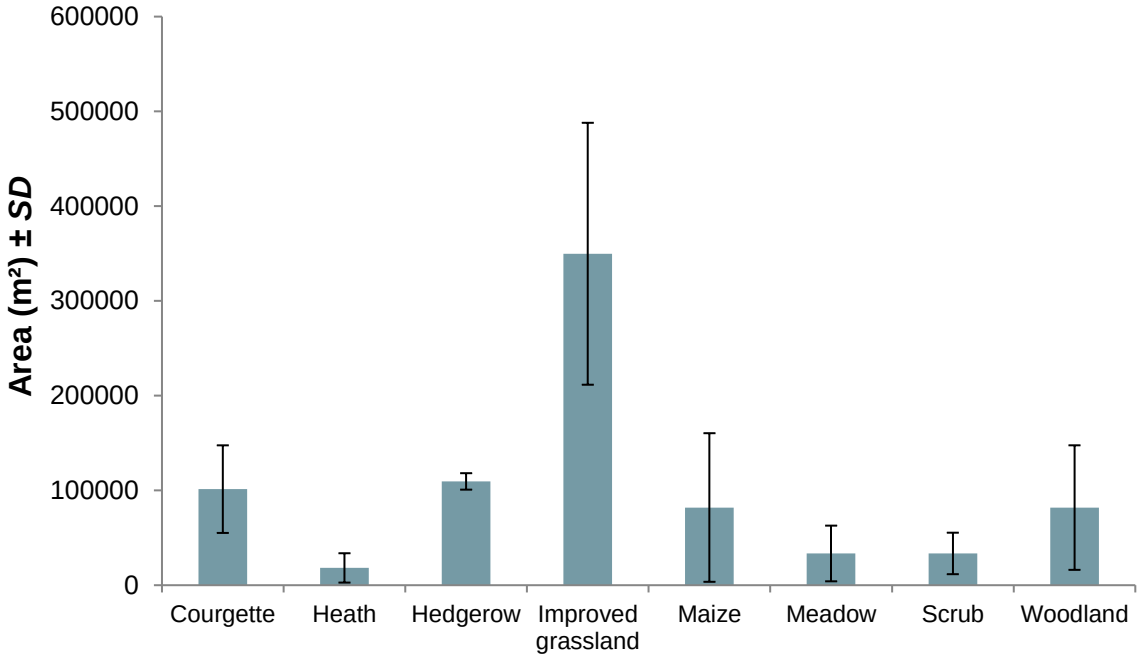

Figure A 4.1 Area (m²) ± *SD* of the different habitat types providing pollen and/or nectar across the 14 landscapes. In model simulations the area of courgette was specified as either 'early courgette' or 'late courgette' (see Table A 4.1), or 'no courgette' (no habitat specified in the model). *Simulated B. terrestris* were able to nest in heath, hedgerow, meadow, scrub, and woodland.

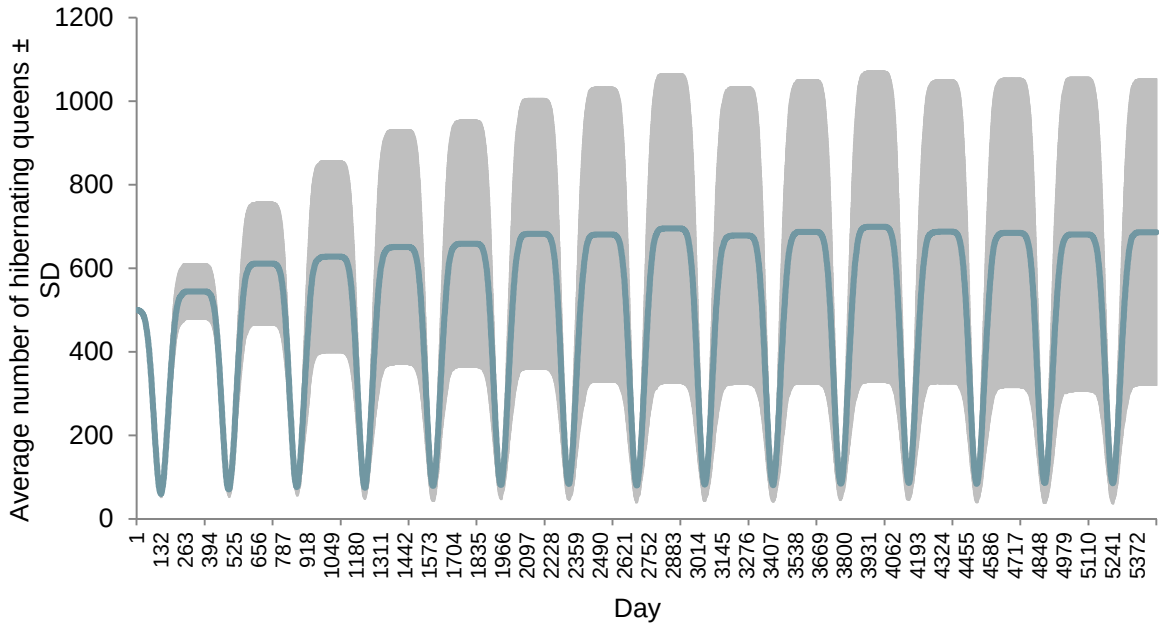

Figure A 4.2 Average number of hibernating queens ± *SD* (shaded) across 14 landscapes with no courgette present (baseline) over 15 years. Data were simulated 20 times for each landscape.

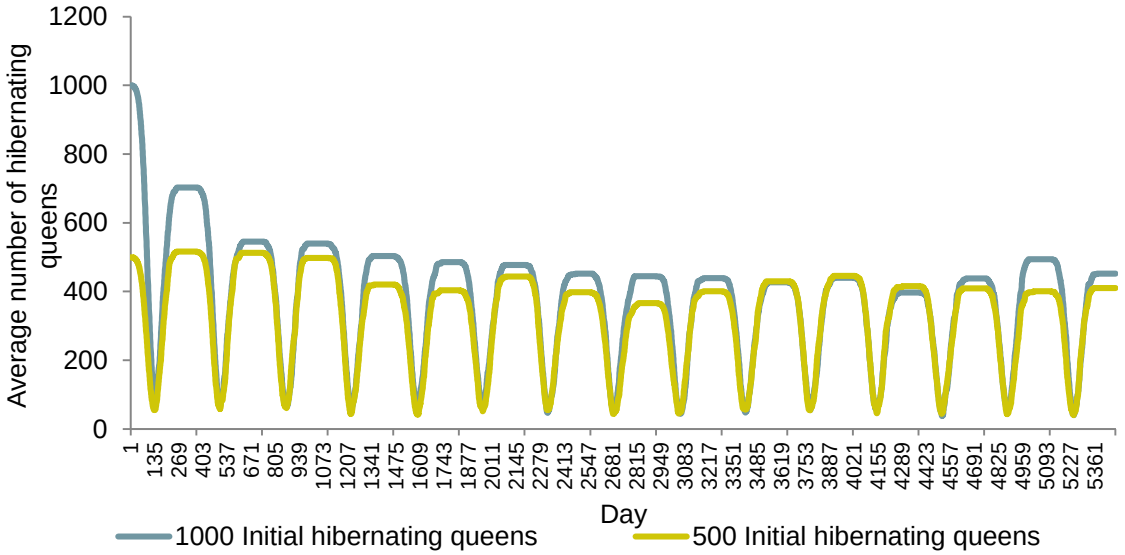

Figure A 4.3 Average numbers of hibernating queens simulated for the study site 10, the last of the 14 different landscapes to reach equilibrium, i.e. when both growth curves have converged; taken as year 11 (a conservative estimate). Data were simulated 20 times for each landscape.

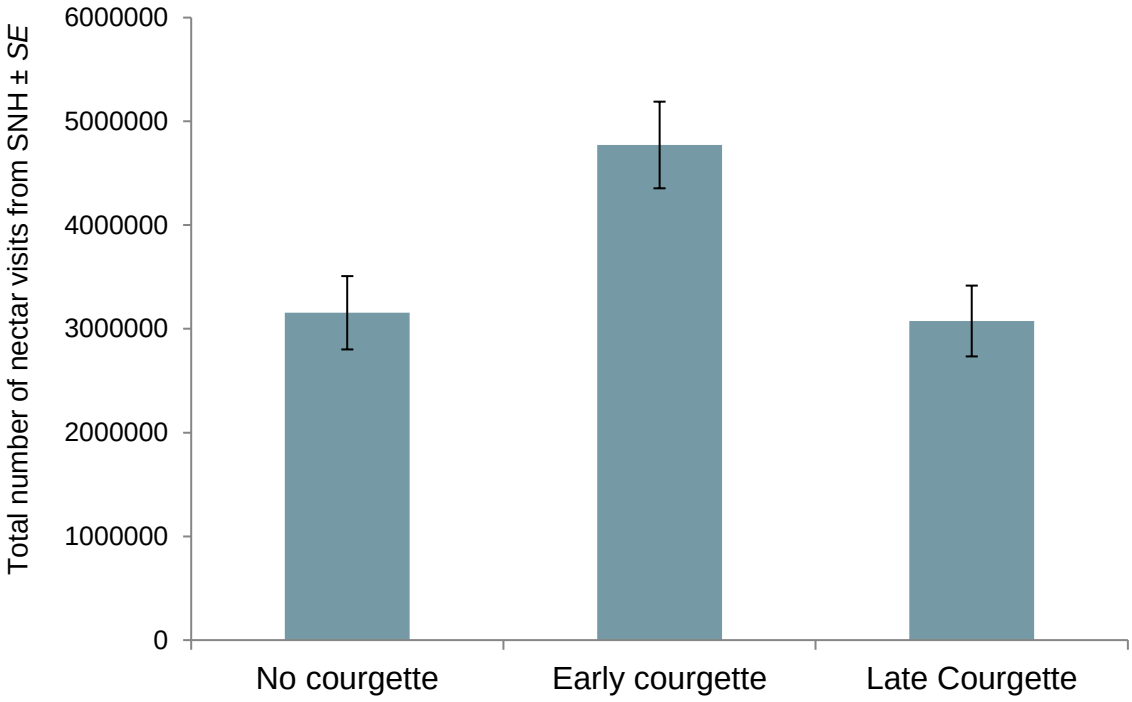

Figure A 4.4 Total simulated number of forager visits ± *SE* at the end of the year (year 11) to patches of SNH, semi-natural habitat: heathland, species-rich grassland, hedgerow, scrub and woodland for nectar in each of the three cropping scenarios. Data were averaged across the 10 repeated runs and 14 study sites.

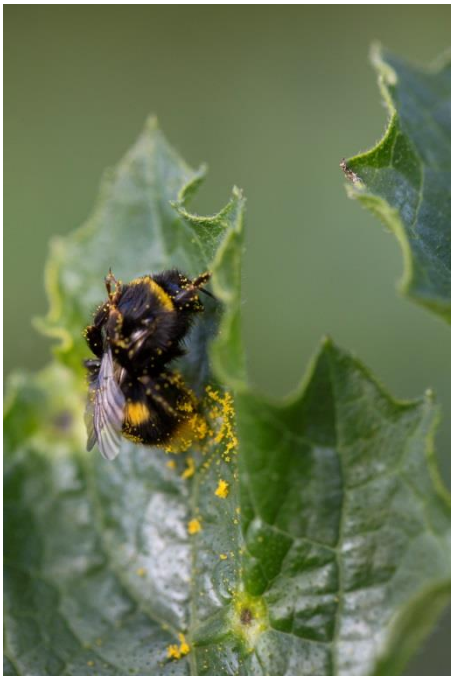

Figure A 4.5 *B. terrestris* removing excess courgette pollen from its body.

Appendix 5. Thesis discussion

5.1 Effect of introduced B. terrestris colonies on courgette yield

In 2017, the effect of supplementing colonies of *B. terrestris* was investigated by comparing the yield of courgettes when colonies were open to when colonies were closed. One field (2.58 Ha) in Cornwall was stocked with six commercially reared *B. terrestris* colonies (Biobest Biological Systems, Belgium), near to the recommended stocking density for courgette (Koppert Biological Systems, Netherlands).

Colonies were opened or closed on alternate sampling days from the 1st August to the 7th September. On days where colonies were open bees were free to forage, but on days where colonies were closed, bees were contained within their colony. The afternoon before bees were contained the standard entrance was changed to one with a one-way valve so that any foragers already outside the colony could return, whilst preventing any additional bees from leaving the colony. Each colony contained one reproductive queen and around 80 workers, and was provided 1.4 litres of sugar syrup for additional nutrition, as recommended by Biobest. Since colonies were only closed for one day there was no need to provide additional pollen. Colonies were insulated and waterproofed with polystyrene and placed inside metal cages which were pegged to the ground to reduce the risk of predation from badgers.

Since courgette flowers are monoecious, pistillate flowers were individually monitored (identified with marker pen written on pieces of flagging tape, tied to the base of each fruit) to quantify yield from plots randomly located within the field. This was done with *B. terrestris* colonies open (n = 100) and closed (n =1 00) over 10 sampling days (20 flowers per day). Fruits were then harvested 10

days post-anthesis, weighed on scales and measured using a tape to determine their length. To avoid the confounding effect of a plant investing in additional fruits from un-monitored pollination events, only one fruit per plant was studied (Stephenson *et al.* 1988, Avila-Sakar *et al.* 2001). Experimental fruits were classed as 'aborted' if they did not meet minimum commercial standards (Ellis Luckhurst, personal communication 24th June 2015), i.e. they were less than 14 cm long, 30 mm wide (at the mid-point), and over 5° in curvature, or showed any obvious signs of bacterial damage, such as blossom end rot. Therefore, fruit set (the ratio of marketable fruit compared to the total number of marked flowers per treatment) is also a measure of fruit quality. As fruit set was measured over 10 days courgettes were generally larger than commercial standards. Since these experiments were conducted at a commercial farm some fruits were accidentally removed by pickers. Consequently, final sample sizes were less than the number initiated and are not completely balanced between treatments (with *B. terrestris* n = 97, without *B. terrestris* n = 96).

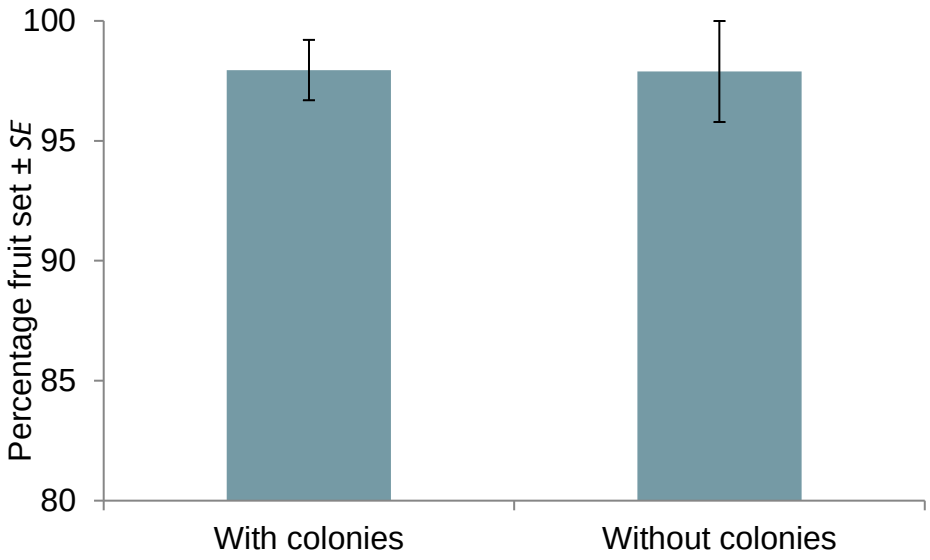

Figure A 5.1 Percentage fruit set with and without commercial *B. terrestris* colonies open. Data were combined for the 5 days when colonies were open and the 5 days when colonies were closed.

There was no difference in courgette yield when *B. terrestris* colonies were open, compared to when they were closed (Figure A 5.1). This is likely because of the already high level of pollination at study sites which is indistinguishable from natural fluctuations in yield.

Appendix 6. Engagement with growers

PRODUCING **PERFECT PRODUCE** BY PARTHENOCARPY

Can the traditional methods of pollination in cucurbits be bypassed to create perfect produce via parthenocarpy? Jessica Knapp, University of Exeter, investigates

It is no secret that, like most plants that produce a fruit, Cucurbit crops generally require pollination from insects for their fruit to be large and well formed.

However, the process requires co-operation from the insects and sometimes considerable work from the grower to ensure that each plant achieves the pollination required.

So, if there was an 'easier' way to ensure cucurbits achieved marketable size then it would be a significant boost to growers everywhere in terms of savings on pollinators and labour.

This is where 'parthenocarpy'– the term used for fruit set in the absence of fertilisation – becomes of interest because of its potential to help cucurbits to reach maturity via the use of selective breeding.

AHDB project 'CP 118 Cucurbit pollination: mechanisms and management to optimise field crop quality and quantity' set out to investigate if pollination is limiting to fruit quality and quantity, and if so, under what environmental conditions, in courgettes (variant: Tosca).

Initial findings showed that while pollination increases the size, weight, and growth rate of courgettes, natural parthenocarpy means that many fruits were able to reach marketable size and shape without any pollination at all.

Results of a literature review showed that all techniques to induce parthenocarpy (selective breeding, hormone application, or genetic modification) were able to increase fruit quantity without adversely affecting quality in 18 pollinator-dependent crop species (not including seed and nut crops as parthenocarpy causes seedlessness) which traditionally require pollination for fruit set.

Since a crop's need for pollination could be greatly reduced, parthenocarpy could allow producers to extend their growing seasons to exploit environmental conditions usually adverse for pollinators, furthering their economic advantage, increasing agricultural resilience, and improving food security.

Despite the natural parthenocarpic tendency of courgette, no selectively-bred parthenocarpic varieties are currently grown at a commercial scale in the United Kingdom. Pollination experiments show that 41% of fruit set is still dependent on natural pollination, with percentage fruit set, the size and weight, but not sugar content, of courgettes being significantly increased with pollination.

Fortunately, at most of the study sites the pollination rates were so high that there was no statistical difference in the yield of hand (artificial) and open-pollinated (insect pollinated) fruits. This may explain why, when commercial bumblebee colonies were introduced, there was no difference in yield (percentage fruit set, weight, or length) between fields with or without colonies.

Of course, pollination is entirely dependent on the availability of pollinators and suitable habitat to sustain their populations. Therefore, the high level of pollination observed in this study is likely to vary between courgette-growing regions. For example, our study across different sites in the UK showed that more wild flowers in field margins resulted in more pollinators in courgette fields. Therefore, allowing uncultivated areas around the crop to be colonised by wild flowers could be an effective way of boosting pollinator visitation, which in turn may increase yield.

Since the total economic value of insect pollination to courgette is estimated to be worth £2.7 million in the UK (£3,400 per hectare), growers may wish to preserve their wild flowers on the edge of fields as a way of mitigating potential fluctuations in pollinator populations and yield. In doing so, they may also enhance other ecosystem services vital for agricultural production, such as natural pest control.

"Many fruits were able to reach marketable size and shape without any pollination"

AHDB project code: CP 118
Project lead: Jessica Knapp, University of Exeter
AHDB contact: Grace Choto

Figure A 6.1 Article published in The Grower, AHDB's technical journal for horticulture. Issue No. 239, Apr/May 2018.

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